

A STUDY OF FEEBLE BIO-ELECTRIC CURRENTS BY MEANS
OF THERMIONIC VALVE AMPLIFICATION.

By

James Yule Bogue, M.R.C.V.S.,

Presented for the degree of Ph.D., in the
University of Edinburgh.



CONTENTS.

	<u>Page</u>
I. Introduction —————	2
II. Technique —————	5
III. Electrical Response of the Chick Embryo Heart —	26
IV. The Electrocardiogram of the Chick —————	46
V. The Electrical Response of Some Marine Hearts —	52
VI. Discussion —————	60
VII. Summary —————	76
VIII. References —————	79

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I. INTRODUCTION

Acting on a suggestion made by Professor Crew that there might be a difference in the heart rate of the male and female chick embryo, an attempt was made to devise a method by which it would be possible to obtain an electrical record of the heart beat with the minimum of interference in order to get the true rate of the heart. After several attempts records were obtained using valve amplification showing the gross form of the electrocardiogram of the embryo at a comparatively early stage. It was therefore thought advisable to investigate the question more carefully to see if it was not possible to evolve a technique that would give electrocardiograms of developing and primitive hearts with a minimum of distortion^{and} with as simple a method as possible.

Considerable difficulties were encountered in attempting to record these small action currents, as they are of such small magnitude that very often the results obtained in the usual manner are poor and unsatisfactory owing to the number of errors that/

that can arise in using a sensitive instrument at its maximum sensitivity. Such records as are obtained by means of the Einthoven galvanometer involve the use of a very slack string; this is not desirable for the following reasons: (a) the string may easily be broken, (b) it is very sensitive to external vibration which may mar the record, and (c) it may also be abnormal in form owing to the slackness of the string with a tendency to overshooting. The electrodes in this case must be placed directly upon the heart, which is quite permissible in a large number of experiments, but in the case of a very delicate developing heart (e.g. the chick embryo) there are several disadvantages: at about 35 hours it is possible to obtain an electrocardiogram, but if the electrodes be placed upon the heart, there is considerable risk of damage, the slightest touch with the electrodes damaging the heart and causing an abnormal response or even arresting the beat in many cases. Sometimes the mere exposure of the heart results in an abnormal response owing to temperature variations.

Since the Einthoven galvanometer is a very sensitive instrument with only a small amount of inertia/

inertia, the amount of energy required to obtain a response is very small. Einthoven (1) stated that the amount of energy required to give a visible movement of the string corresponded to that expended in lifting one hundredth of a milligramme to the level of one millionth part of a micron. This fact is important since it means that whatever method is employed to get an amplified current, it is unnecessary to consider the power factor. The elimination of a power stage in an amplifier does away with one of the sources of distortion. This confines the requirements of the amplifier to a high amplification factor with stability and no distortion.

II. /

II. TECHNIQUE.

This research was commenced under considerable difficulties, the B.B.C. transmitter aerial being only about 100 feet from the laboratory. This resulted in a considerable amount of interference and it was only possible to work between midnight and about 5 a.m.; even then it was not always possible to avoid interference since there were other sources, such as electrical apparatus that was continually in use. The laboratory was also subject to vibration from traffic in the street and being situated in the roof, earthing facilities were unsatisfactory, the resistance to earth being very high. Fig. 1 shows an attempt to take an electrocardiogram of a chick embryo during the day.

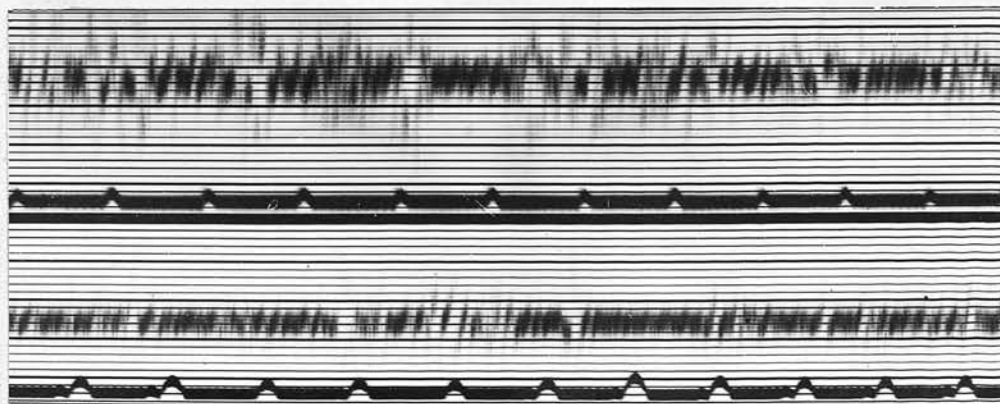


Fig. 1.

Advantage/

Advantage was taken of the structural alterations in the University to have a special room designed for pursuing this type of research with the minimum of interference. It perhaps merits a brief description here as all interference was cut out. The room (see Fig. 2) is 18 x 10 feet with a reinforced Klein concrete floor to stand 25,000 lbs. in weight; the floor, ceiling and walls were lined with expanded metal lath, each section being welded together. All electrical conduits were outside the lath, and holes were left in the lath for the switch boxes and wall plugs which were electrically connected to the lath. This was covered with concrete plaster and the whole was covered with copper gauze of a very fine mesh, the upper half of the window being permanently screened off and the lower half screened by means of a removable wooden frame covered with gauze; the door was also covered with gauze with brass flanges around the edges so as to ensure contact with the metal on the walls when closed. The copper on the floor and ceiling was soldered together along its whole length, while the strips on the walls were connected with channelled lead(as used for stained glass windows) by placing the copper in the channels on each/

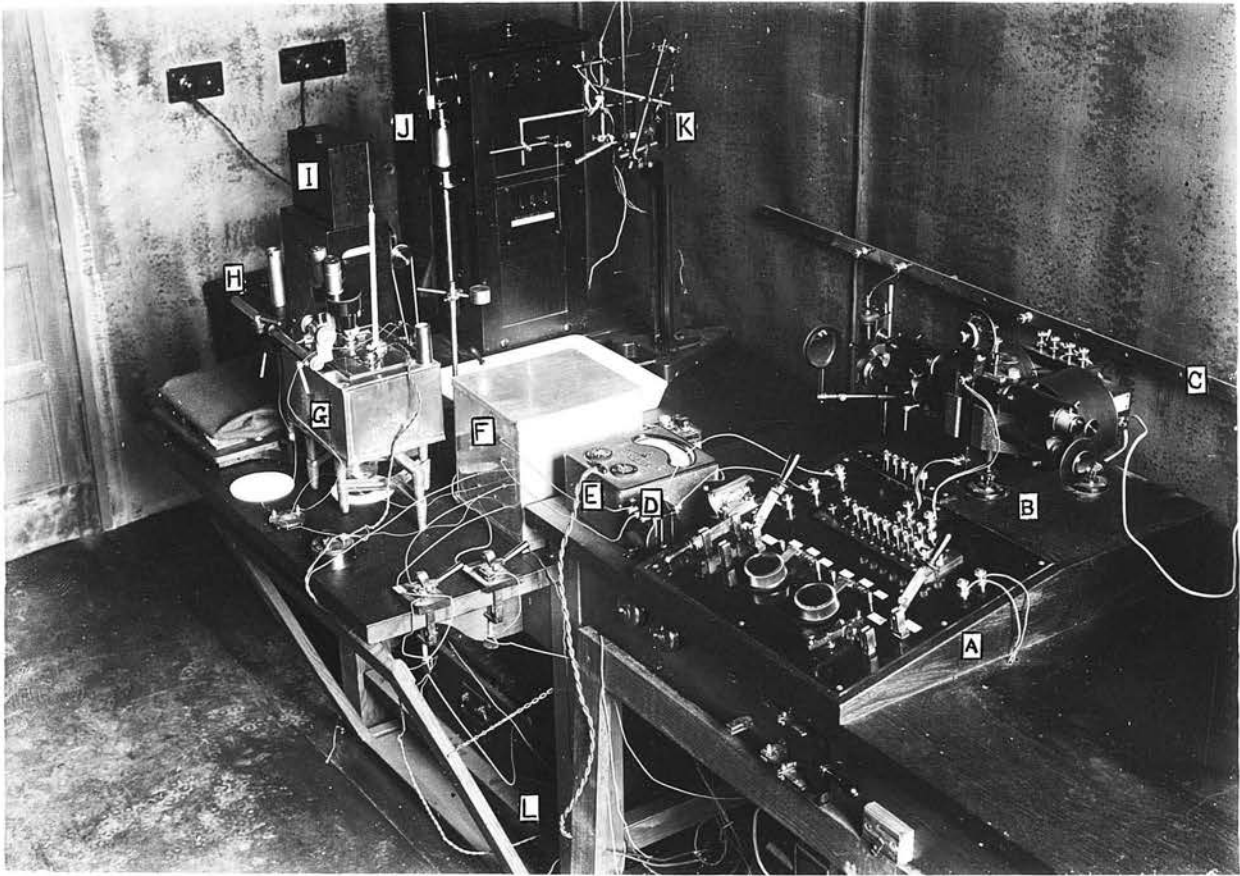


Fig. 2. Galvanometer Bench. (a) Control panel, (b) galvanometer, (c) earth strip, (d) l/l transformer, (e) test meter, (f) screen grid amplifier, (g) egg chamber, (h) corner of incubator, (i) bromide camera, (j) plate camera, (k) electrode and mechanogram stand, (l) battery trays and folding rails.

each side of the lead strip and hammering it down.

A galvanometer bench of one and a half inch teak was constructed so as to have everything as steady as possible. Underneath the bench were teak trays on roller bearings which ran on wooden rails. These carried the batteries of which there were about 400 volts altogether. The whole system was earthed by means of a heavy copper strip leading to the ground. The arcs for the optical system of the galvanometer were housed in a gauze cage and the mains lead from the power plug was metal sheathed. No other mains were on during an experiment, any light that might be required being obtained from battery driven bulbs; all external electrical interference was thus eliminated. To test the efficiency of the screening a 9 valve wireless set was connected up in the room, and it was impossible to tune into the local transmitter only 30 yards away even with the door open unless 1-2 feet of aerial was outside the door.

Valves.

A large number of different valves were tested in suitable circuits in order to find out which would fulfil the requirements. The valves tested fell into three main classes, namely, triodes/

triodes, pentodes and screen grids.

Triodes - These are adaptable for high frequency magnification, rectification and low frequency amplification, the main difference between the different types being in the size of the mesh of the grid and in the placing and shape of the electrodes. The characteristics may vary as follows:

Amplification factor.....	3.5-40.
Anode A/C resistance.....	1,300-90,000 ohms
Mutual A/C conductance.....	2.7-0.45 Ma/V.

These represent the two extremes, the former P.650 being a valve of the super-power class which does not concern us here and the latter, an H.670 resistance capacity coupling valve for L-F amplification.

Pentodes. - The main advantages of this type of valve is that they have a fairly large amplification factor (up to 80) and power output for a reasonably small grid swing, but they have the disadvantage for the work in question that they are unsatisfactory when coupled together in cascade. Difficulty was encountered in trying to keep the amplifier stable under such conditions, but if a pentode was used in the output stage and triodes in the preceding stages, stability was quite easily maintained.

Screen/

Screen Grid Valves - These valves are primarily designed for high frequency amplification in radio work and have a high magnification factor with a very small power output and small current consumption. With the advent of the indirectly heated cathode screen grid valve, an amplification factor of 1,200 was available, the highest amplification factor obtainable with the directly heated cathode being 300.

Designing the Amplifier.

Three stages of amplification were employed as four or more usually involved certain losses in sensitivity which caused distortion of the curves unless elaborate precautions were taken.

There are three choices of coupling, namely:

(1) Transformer, (2) Choke, and (3) Resistance Capacity Coupling.

(1) Transformer Coupling. Although very good for ordinary low frequency amplification it was not found to be suitable for this work. A three valve amplifier incorporating triodes and 3.5 to 1 intervalve transformers was constructed, but owing to the distortion of the curves of the electrocardiogram/

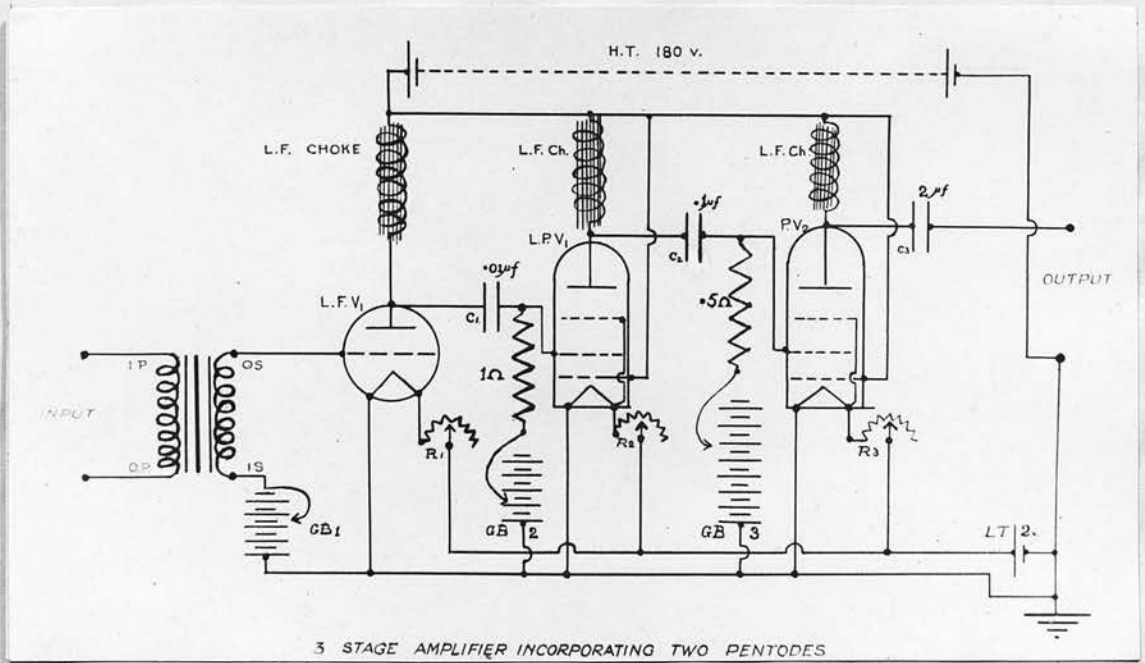


Fig. 3. Circuit diagram of choke coupled amplifier. The input transformer was omitted for this work.

cardiogram, no further investigation was carried out along these lines.

(2) Choke Coupling. A three valve amplifier built according to the circuit diagram in Fig. 3 was constructed. Records were obtained by using three simple triode valves, but the sensitivity was not great enough for the very early stages of the developing embryo. A record obtained under these conditions is shown in Fig. 4 . The output valve was then replaced by a pentode which markedly increased the sensitivity (Fig. 5). With a triode in the first stage and pentodes in the following stages, a very high amplification factor was obtained, but the whole was very unstable (Fig. 6).

Two forms of distortion were evident in each of the foregoing combinations of valves - the T wave was always eliminated and the R wave was polyphasic. The pentodes showed a tendency to be microphonic even though the amplifier was mounted on rubber sponges and the valves encased in cotton wool.

(3) /

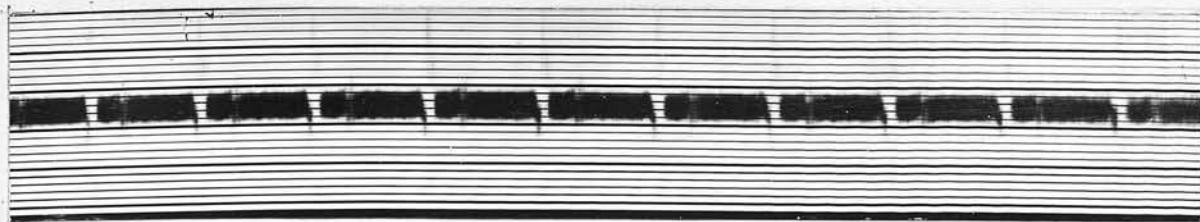


Fig. 4. Type of curve obtained when employing three triodes with choke coupling. Hen embryo, 8th day. In this and succeeding records read right to left unless otherwise stated.



Fig. 5. Type of curve obtained when employing two triodes and pentode output, choke coupling. Hen embryo, 8th day. Note embryo movements in record (b).

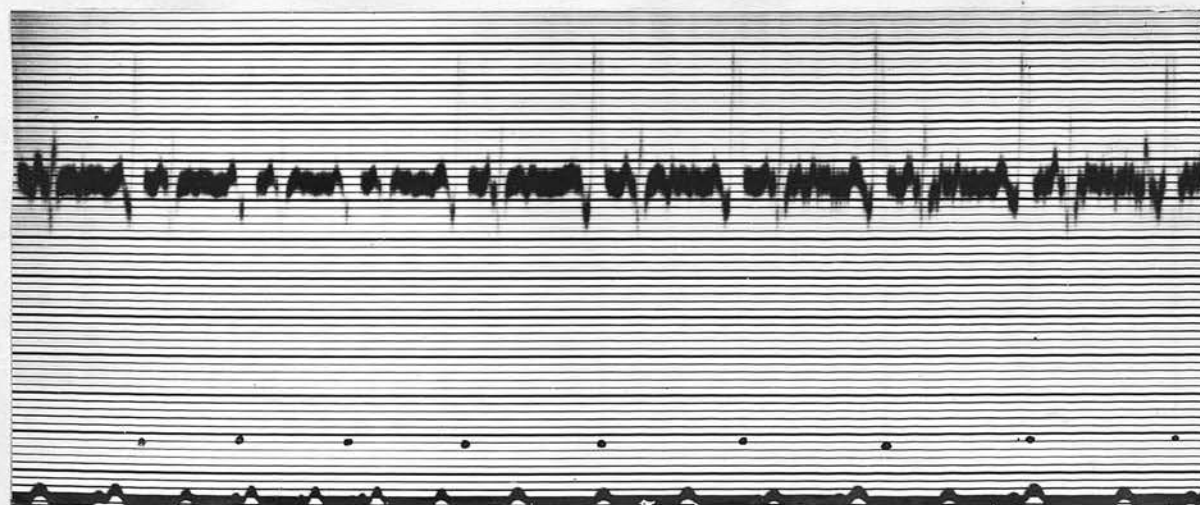


Fig. 6. Type of curve obtained when employing one triode followed by two pentodes in cascade, choke coupling. Hen embryo, 10th day. Tips of R wave shown with dots.

(3) Resistance Capacity Coupling.

This type of coupling gave much the best results in multivalve amplifiers and also has the advantage that it is easy to limit the amplification to a narrow frequency band, by a suitable choice of the coupling components. A three valve amplifier as employed by Adrian was constructed which gave satisfactory results (Fig. 34.). With the directly heated filament valves there is a fluctuation in the electron emission known as the Schottky effect. Yates-Fish (2) noted that irregularities in the amplifier were due to variations in the potential of the first grid, since they were reduced by increasing its capacity and removed by earthing it. The Schottky effect was therefore responsible and must operate by causing variations of the grid current about its zero mean value. A valve with an equipotential cathode would be an obvious improvement.

The AC/Screen grid seemed to offer the solution. Owing to its high magnification factor only one valve is needed and all losses due to coupling one or more valves together were thus eliminated/

eliminated. The Mazda AC/SG was the only valve available and a section made from one that was accidentally broken is shown in Fig. 7 . This is a screen grid valve built for high frequency amplification in which the inter-electrode capacity has been reduced to a negligible amount by means of an internal screen between the grid and the anode of the valve. The valve is designed to be fed from the AC mains, but as the heater consumption is only 1 ampere at 4 volts, a large capacity accumulator can be used. There is no filament as in ordinary valves but a tubular cathode which surrounds the porcelain coated heater. The non-inductive heater is bent into the form of a hair-pin which is dipped into a liquid porcelain slip so that it is coated with an adherent insulator. This is inserted into a metal tube coated with oxides of barium and strontium. The cathode is of small diameter and of considerable length; the watts dissipation per cm. is small so that the control grid is kept cool and grid emission is prevented. The cathode has an equipotential surface (i.e. no potential gradient as in ordinary filaments) and no field around it, so that the electron flow is less restricted than in the/

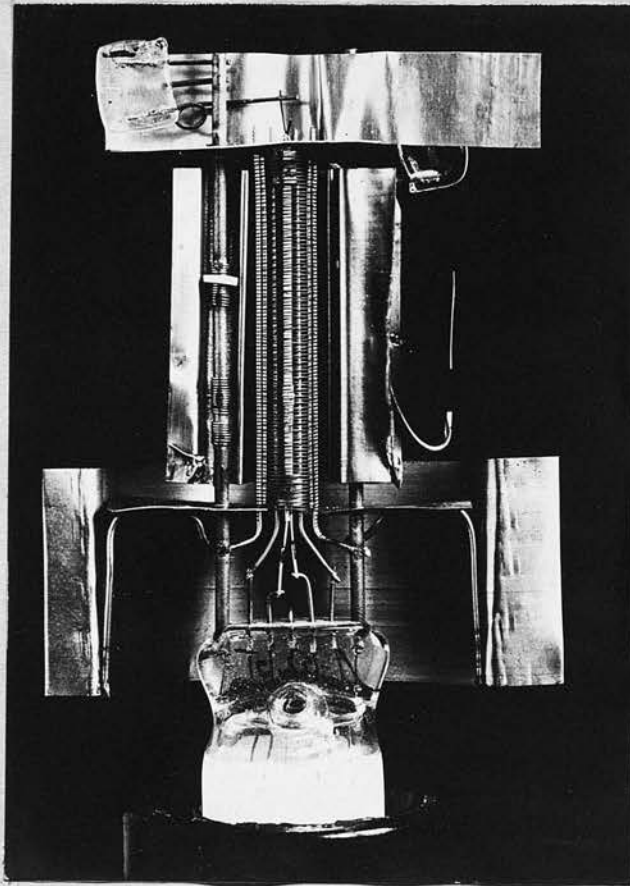
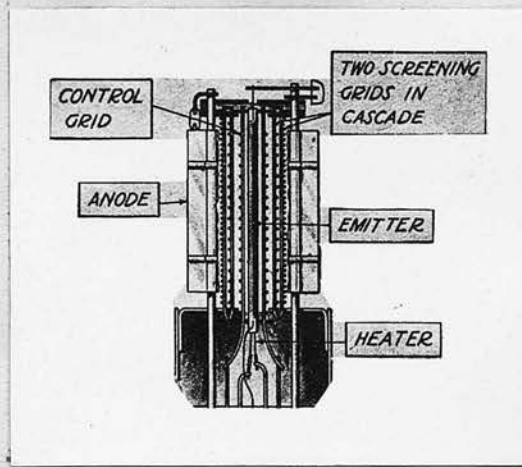


Fig. 7. Photograph and diagram to show the construction of the Mazda AC/SG valve. Compare with Figs. 8, 9 and 10.

the ordinary valve.

Around the cathode in the form of a fairly widely spaced spiral is the control grid; outside the control grid are two screening grids in cascade, which consist of two spirals one within the other and about 0.05 cm. apart. These are continuous with the cylindrical and transverse metal shields just above the glass pinch and also with the two small metal shields at the top of the valve. Outside the screening grids is the anode, which is a sheet of metal arranged closely around the grids and metal supports and is shaped so as to follow their contour. Attached to one side of the anode is a small plate upon which is the substance for gettering the valve after pumping.

Specification. (As given by the makers).

Filament volts	_____	4.0
Filament amps. (approx.)	_____	1.0
Anode volts (max.)	_____	200
Effective inter-electrode capacity (cms)	_____	.0045
Amplification factor (m)	_____	1,200
Mutual conductance (G in mA/volt)	_____	1.5

m and G above are measured at $E_a = 150 \text{ V. } E_s = 60 \text{ V. } E_g = -1.5 \text{ V.}$

The/

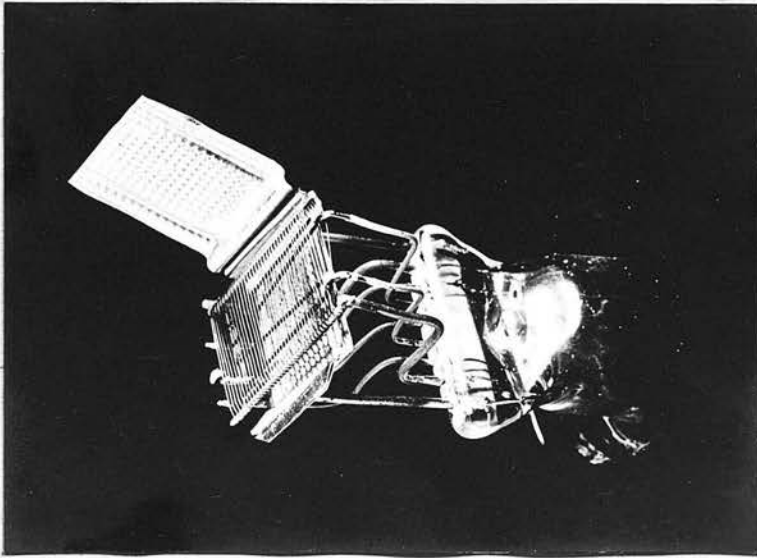


Fig. 8. Typical triode (PM5B), anode bent back to show grid and filament.

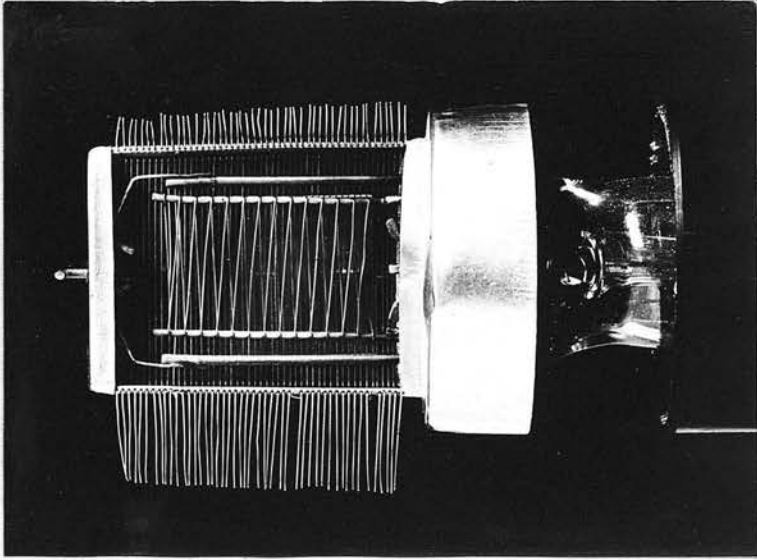


Fig. 9. Battery heated filament screen grid (PM16), filament missing, one plate of anode removed. Note wide spacing of electrodes.

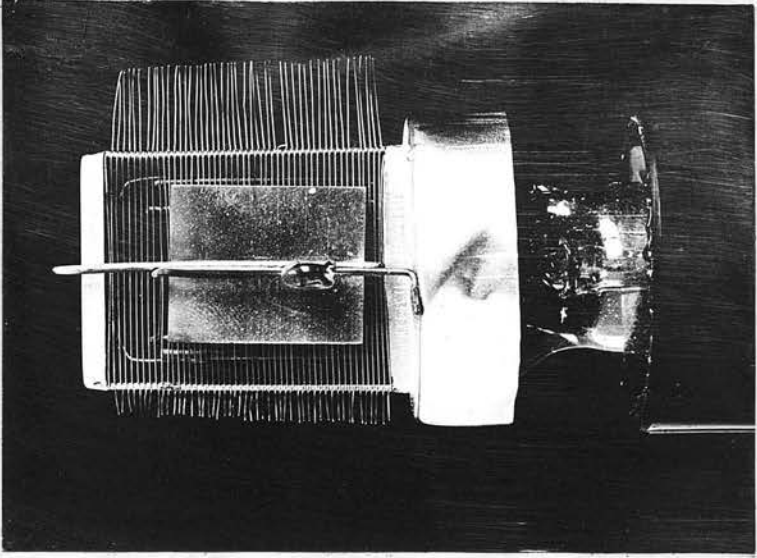


Fig. 10. Rear view of PM16 showing anode in position.

The Amplifier.

The most suitable circuit for use in conjunction with the Mazda AC/SG is shown in Fig. 11. A lead is taken from one electrode to one end of a 400 ohm potentiometer which is across a 2 volt cell, the sliding contact being connected to the control grid of the valve. A 2 megohm grid leak is connected between the grid and the cathode, which is continuous with the other electrode through to one of the output terminals, the high tension negative forming the other output lead. Critical negative bias was obtained by means of the potentiometer; a grid bias battery in series with the grid leak and cathode was not quite so satisfactory.

The heater circuit is quite separate, the current being ^{er}divided from the field batteries of the galvanometer which were of large capacity (85 ampere hours actual), so that variations in the heater temperature due to the battery running down with continuous use were reduced to a minimum.

The screening grid was at a potential of 70 to 80 volts according to the anode voltage employed./

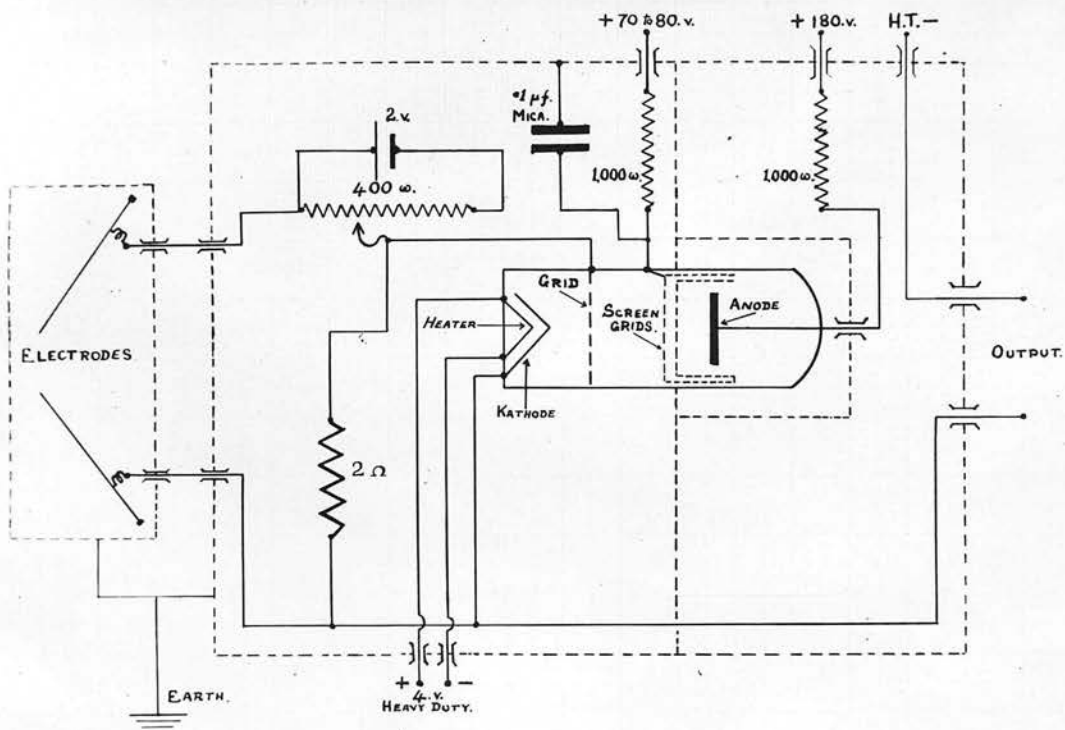


Fig. 11. Circuit diagram of amplifier incorporating a Mazda AC/SG valve, as used throughout this work. Aluminium screening represented by dotted lines.

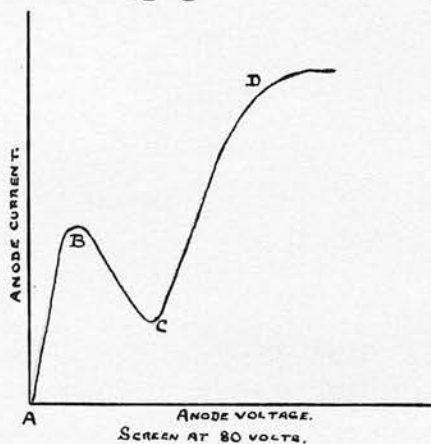
employed. A wire wound decoupling resistance of 1,000 ohms was put in series with the screening grid and battery when using the amplifier in the later stages of development of the embryo, but in the earlier stages (2nd to 5th day) this and the anode resistance were removed and replaced by a copper rod. The anode voltage varied between 150 and 180 volts, according to which gave the best characteristics. A $.1 \mu f$ mica condenser was connected between the screening grid and earth. The whole was screened in an aluminium box, with a transverse screen through which the valve was mounted, so that the lower internal transverse screen of the valve was in the same plane. In order to make the screen as continuous as possible, the hole through which the valve projected was made a little larger than the diameter of the valve. A hole was then cut in a piece of copper foil, the diameter of which was about 1 cm. less than that of the valve at the level of the internal transverse screen of the valve; 40 radial cuts 5 mm. long were then made from the inside of the circle, and the foil was mounted over the hole in the aluminium screen/

screen. A very close contact with the glass of the valve was thus obtained reducing the space between the internal and external screws^{ens} to a minimum.

The amplifier could not be connected directly to the string galvanometer owing to the standing anode current, but this could be balanced out with an equal and opposite current. A milliammeter took the place of the string and a compensator consisting of a 2 volt battery with a zenith tubular resistance for coarse adjustment and a spiral wheatstone bridge for the fine adjustment. The whole was adjusted until the meter was pointing at zero, and the string galvanometer was then switched into circuit, cutting out the milliammeter. It was found that the amplifier worked very satisfactorily with a 1/1 transformer in the output and no compensation. With the transformer the base line was absolutely steady and when opening the short circuit switch of the galvanometer there was no sudden excursion of the string. No difference could be detected between records taken with the compensator in circuit and those taken with/

with the 1/1 transformer in circuit. If a step up transformer was used, there was distortion of the curves in many cases. The amplifier was perfectly safe to use with the galvanometer as long as the high tension switch was not opened or shut without the short circuit switch of the string being closed. The valve and amplifier were tested every week in order to see that no faults had developed. A curve was plotted by varying the grid bias with a constant anode and screen voltage. The mica condenser was also tested as trouble once arose through a small leakage.

Some experiments were carried out using the screen grid valve as a pliodynitron, i.e. at point C. on the accompanying curve, where the potential of the anode is not high enough relative to that of the screening grids.



Though/

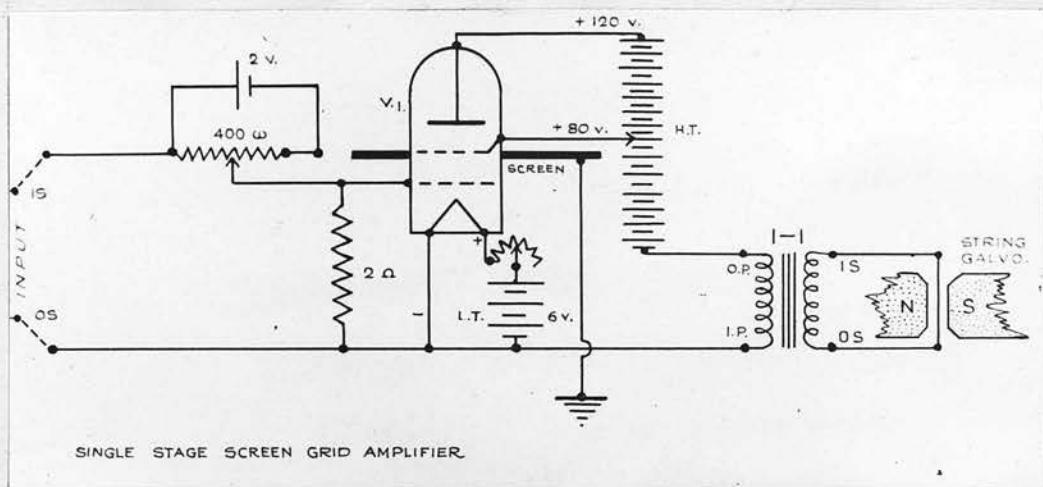


Fig. 12. Circuit diagram of amplifier with a magnification factor of about 60. Used in later stages of development of chick embryo. (After D.T.Harris.)

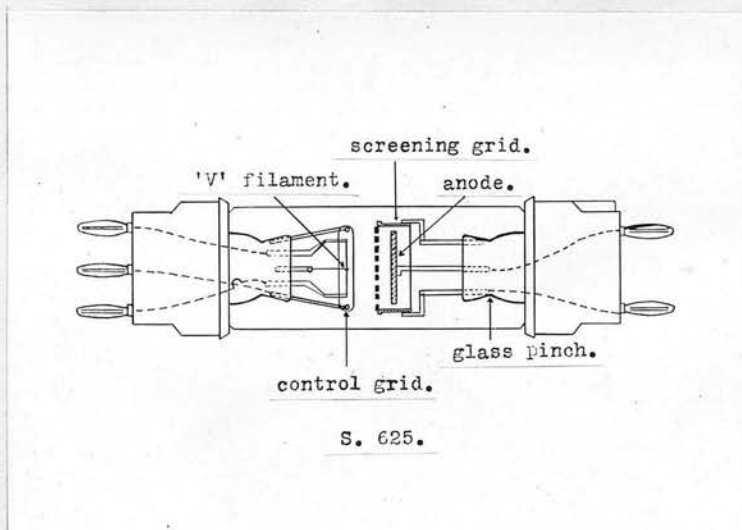


Fig. 13. Diagram to S.625 screen grid valve as used in above circuit. This valve was rejected when the dull emitters came on the market, because being a bright emitter, it did not always run steadily.

Though great sensitivity was obtained at this point, it was found impossible to overcome the distortion of the electrocardiogram. As this method seems to offer great possibilities, further experiments are being carried out on these lines.

III. /

III. THE ELECTRICAL RESPONSE OF THE CHICK EMBRYO HEART.

Procedure.

A warm chamber as shown in Fig.14 was constructed in the following manner: The Chamber was made of copper surrounded by a water jacket, the dimensions being 15 x 10 x 10 cm. The dimensions of the outer box were 4 cm. greater in every direction, the tops of the inner and outer jackets were flush and the space closed over. The inner chamber had a top made of glass through which three holes were drilled, two for the universal joints of the electrodes and one for the thermometer and wires to the candling light. A small water-bath was placed in the chamber so as to keep the atmosphere moist. The chamber also housed the stand for the egg, which consisted of two sheets of vulcanite separated by four vulcanite pillars. An egg shaped aperture, large enough to permit the whole of the embryo to be examined by means of a light underneath the egg, was cut in the top of the/

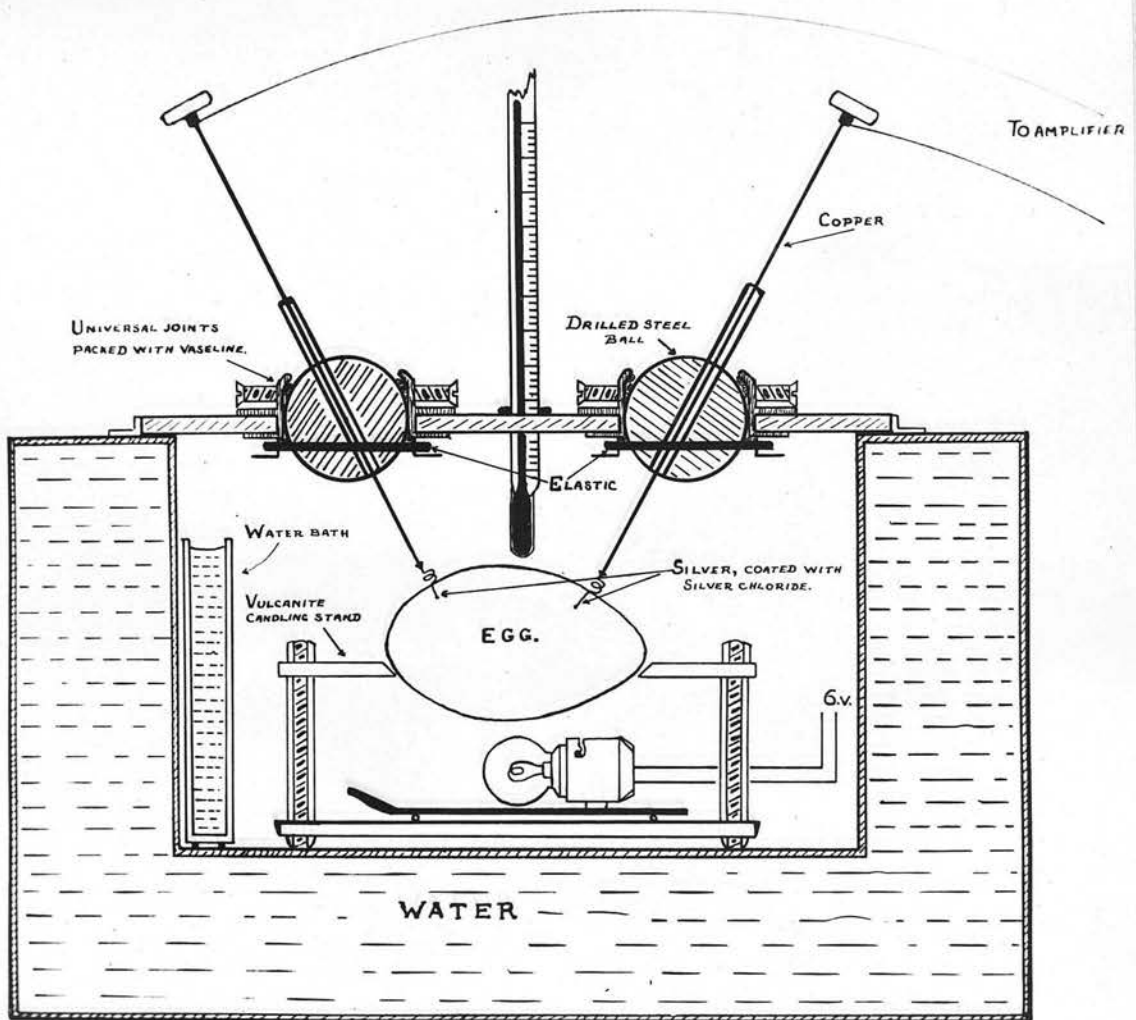


Fig. 14. Diagrammatic drawing of egg chamber.

the stand. The light was supplied by means of a 6 volt torch bulb battery operated and mounted on a polished aluminium reflector.

The electrodes consisted of two large steel ball bearings through which a hole had been drilled; a thick walled fine bore brass tube was secured in each ball, a length of copper wire (gauge 16) was passed through the brass tube, to the distal ends of which the silver electrodes were soldered. At the proximal ends two brass caps, covered with erinoid, were secured to carry the leads to the amplifier. The balls were mounted in a brass case which was clamped on to the glass. The electrodes were secured in position by means of three clamping screws, and free movement was obtained by allowing the balls to rest on a triangle of elastic. This also permitted easy removal of the electrodes for coating or renewal and at the same time prevented them from falling if the screws were loosened too much. The whole joint was well packed with vaseline so as to prevent leakage between the electrodes which could take place through the moisture that condensed on the glass.

The/

The oven could not be heated electrically owing to the interference that would have resulted from the heater element. The temperature was kept constant within a quarter of a degree Centigrade by means of a small ^{spirit}/flame.

The egg to be electrocardiographed was removed from the incubator and candled in order to find the position of the embryo. This was marked with a pencil on the shell, and two small holes were then drilled in the shell, one near to the air sac, the other at the distal end of the embryo. The egg was then placed in the chamber and left for fifteen minutes; then the small light inside the chamber was switched on and the electrodes were pushed through the two holes, piercing the shell membrane, care being taken not to puncture any blood vessels. It was possible to see the shadow of the electrodes through the shell. The universal joints were then clamped and the small light switched out as there was a certain amount of heat radiated from the bulb.

A trial record was then taken to see if the electrodes were in a suitable position; if this was satisfactory, three records were then taken on one plate ^t at five minute intervals. It was found impossible/

impossible to use the bromide camera as the electric motor caused too much vibration and electrical interference. A record taken by the bromide camera is shown in Fig. 15. In the case of embryos from 35 hours to the fourth or fifth day the two holes in the shell were drilled at each end of the embryo and the electrodes were pushed through all the membranes so as to get as near to the heart as possible. It was not possible to obtain satisfactory records otherwise, as the resistance of the egg substance was too great. The main difficulty encountered was the constant movement of the embryo. The electrocardiogram was distorted in two ways: one, by the rotary movements of the embryo which altered the direction of the leads in relation to the heart and made the base line unsteady, and the other by kicking of the embryo. The latter when amplified caused an enormous excursion of the string, especially in the case of a more advanced embryo where the kick was more vigorous. One string was broken in this way. If the amplification was cut down enough to eliminate this danger, it was impossible to obtain a record. The effects of embryonic movements are shown/

shown in Fig. 16. Immediately prior to hatching the respiratory movements caused great distortion of the electrical record, as is shown in Fig. 17.

The collection of a series of records showing the true form of the electrical response of the embryonic chick at various ages was therefore a task of considerable difficulty, which required much patience. In the early stages it was necessary to place the electrodes fairly close to the heart and yet not to touch either the heart or main blood vessels; for a touch caused injury and this altered the frequency. After the electrodes had been adjusted it was necessary, in the case of the older embryos, to wait until the embryo became quiet and then to take a record; this involved long periods of waiting.

The earliest age at which a satisfactory record was obtained was at 33 hours incubation. This record is shown in Fig. 18. It happened that in this case interference from outside causes was at a minimum, and hence this record is exceptionally clear. Figs. 18 to 28 show a series of electrocardiograms taken from the 33rd to the 48th hour of incubation. It will be seen that in/

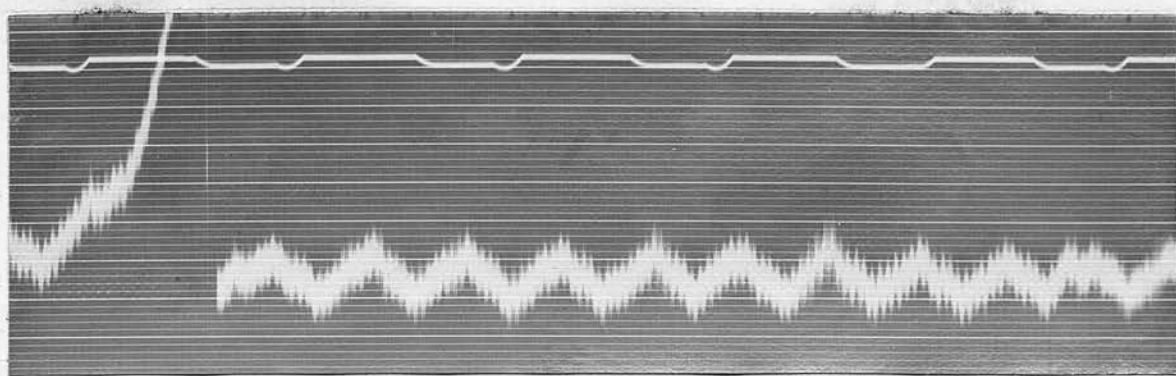


Fig. 15. Interference picked up from the electric motor of driving bromide camera. Note large excursion of string due to the current being switched off.

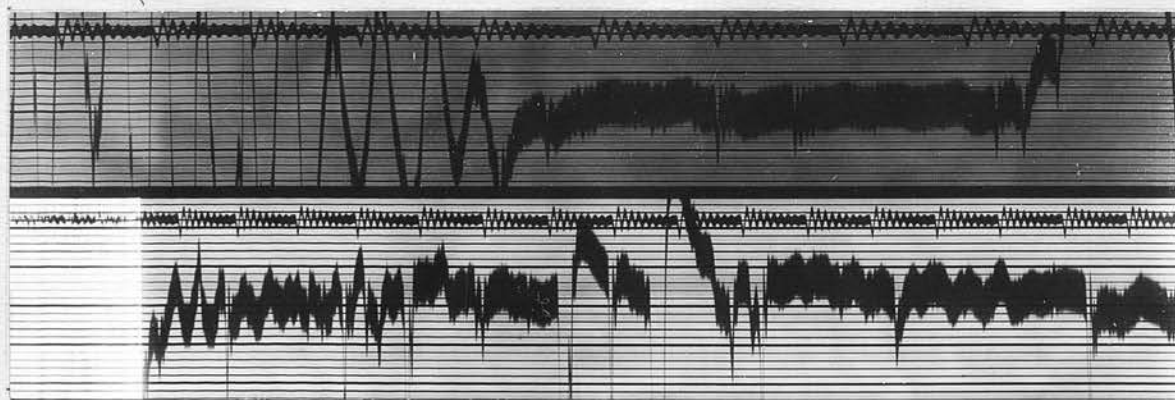


Fig. 16. Distortion of electrocardiogram caused by the kicking movements of the embryo; 15 days of incubation.

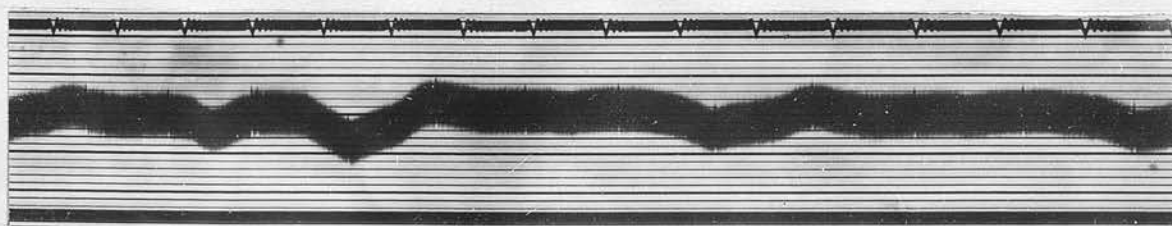


Fig. 17. Unsteady base line caused by the breathing movements of an embryo. The head was just protruding into the air sac. The small rapid excursions show the rate of the heart beat.

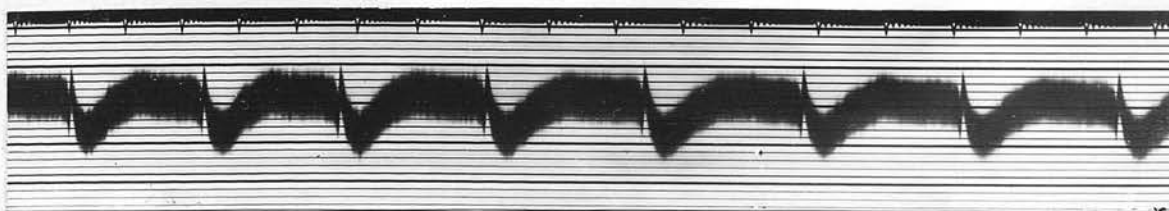


Fig. 18. Electrocardiogram of a 33 hour old embryo.
Time in this and all succeeding records is marked in 0.20 second intervals. Read left to right.

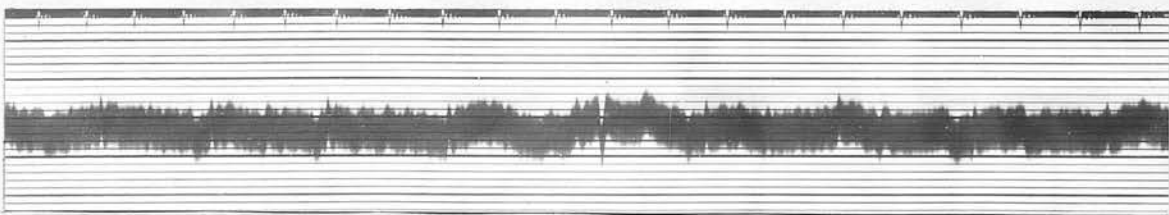


Fig. 19. Electrocardiogram of a 34 hour old embryo.
Read from left to right.

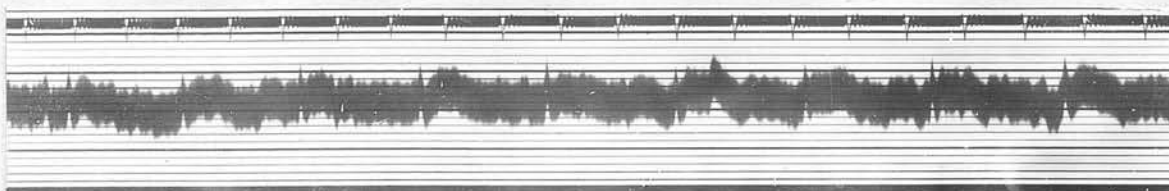


Fig. 20. 35 hour old embryo.
Read from left to right.

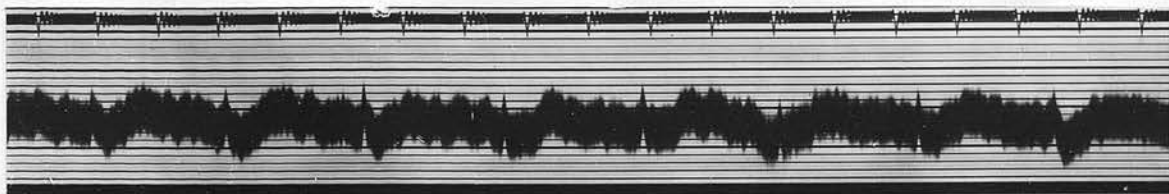


Fig. 21. 36 hour old embryo.
Read from left to right.

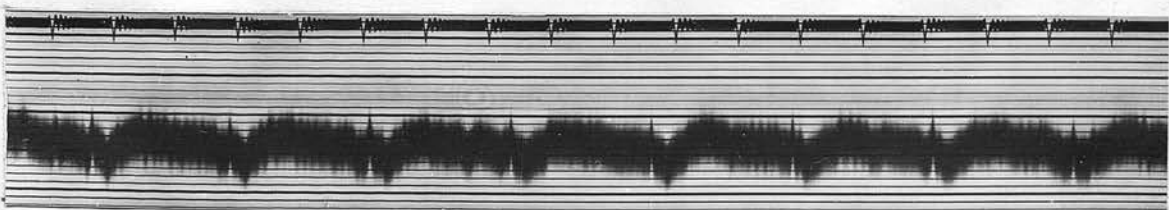


Fig. 22. 37 hour old embryo.
Read from left to right.

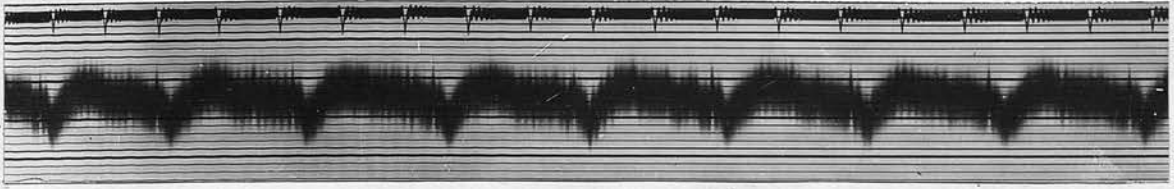


Fig. 23. 38 hour old embryo.
Read from left to right.

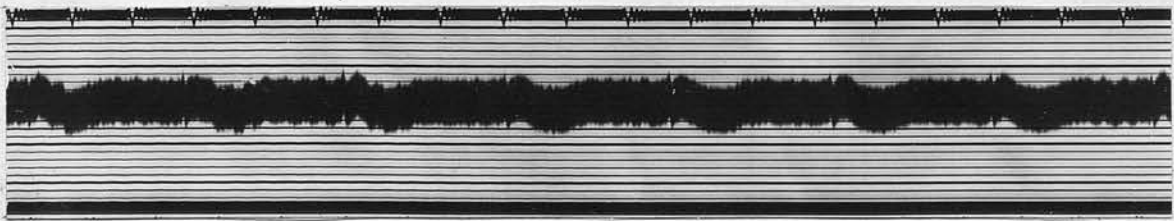


Fig. 24. 40 hour old embryo.
Read from left to right.

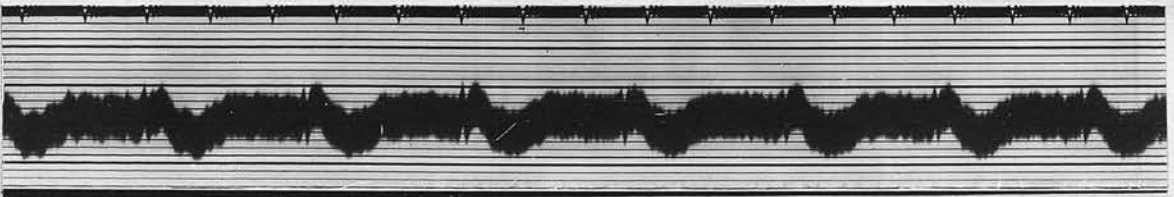


Fig. 25. 42 hour old embryo.
Read from left to right.



Fig. 26. 44 hour old embryo.
Read from left to right.



Fig. 27. 46 hour old embryo.
Read from left to right.

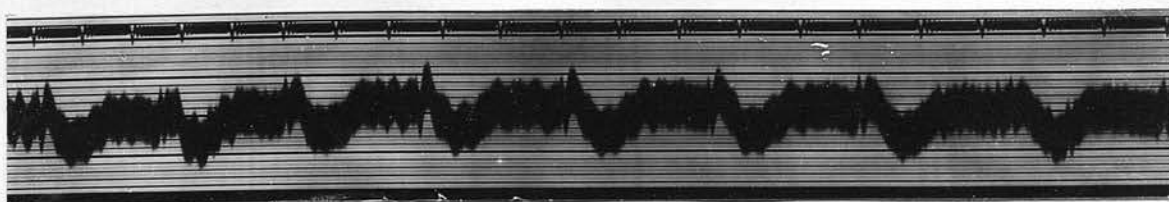


Fig. 28. 48 hour old embryo.
Read from left to right.

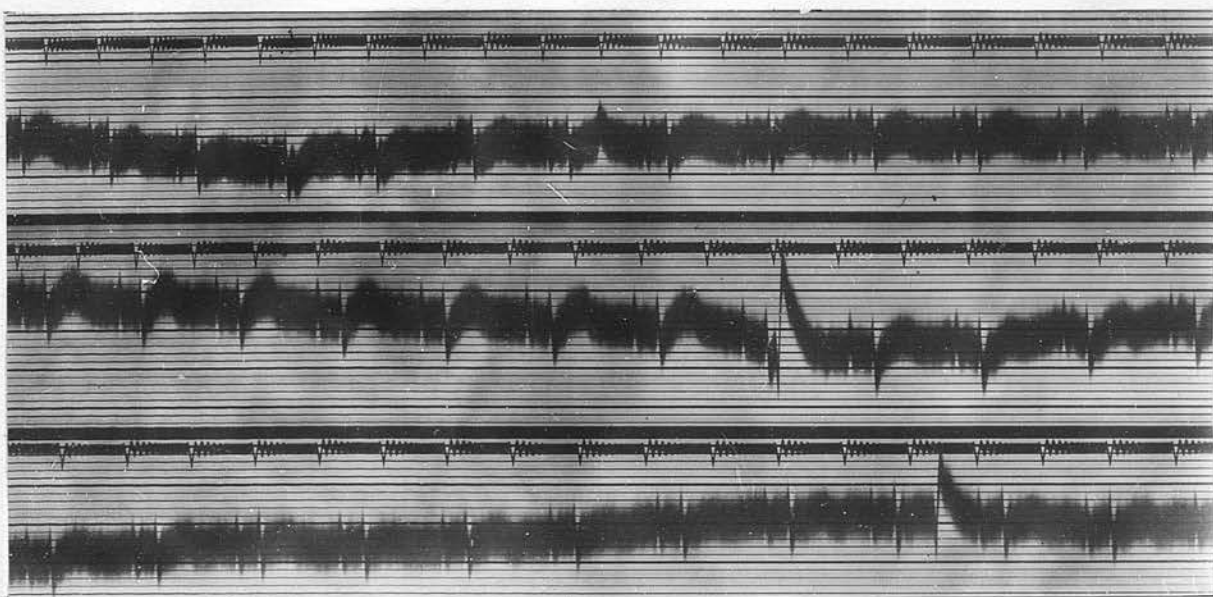


Fig. 29. 52 hour old embryo. This shows the first appearance of the P wave. The electrodes were about 1 cm. apart the embryo lying transversely between them. Read from left to right.

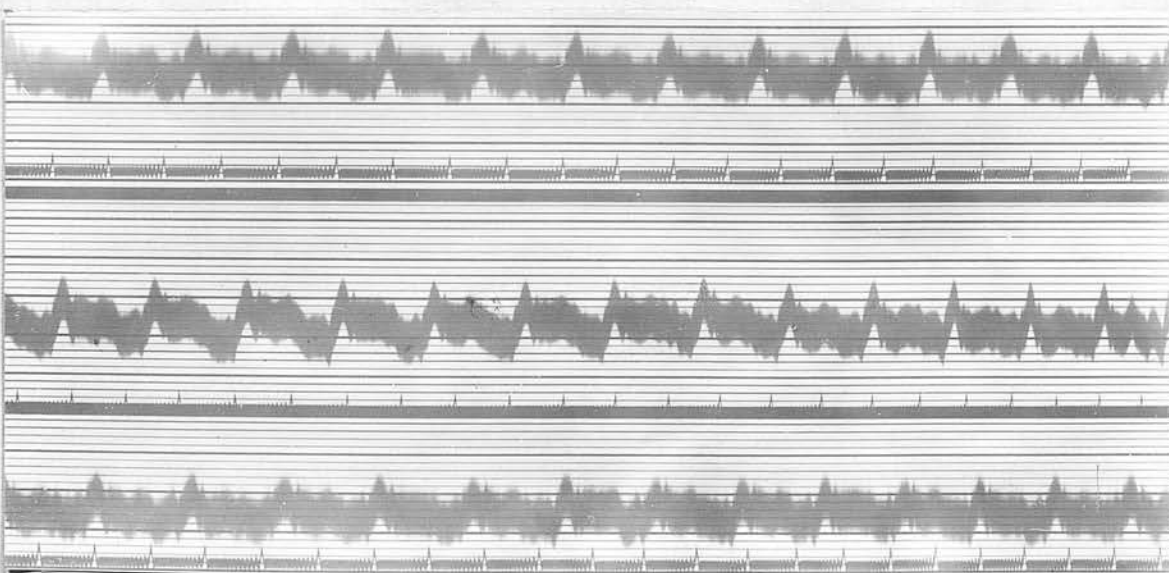


Fig. 30. 3rd. day embryo, exact number of hours uncertain.
Read right to left.

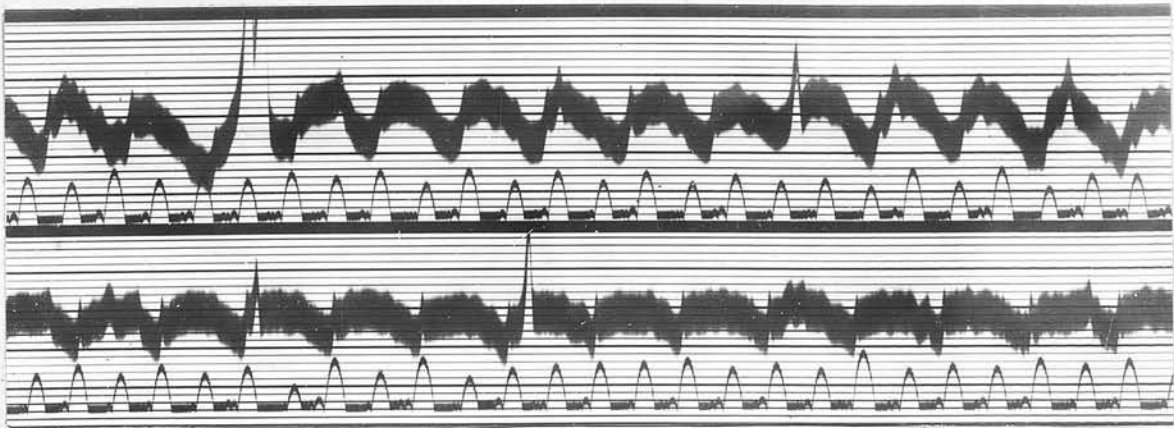


Fig. 31. 61 hour old embryo. Electrodes in this case just pierced the shell membrane and were about 3 cms. apart. Read right to left.

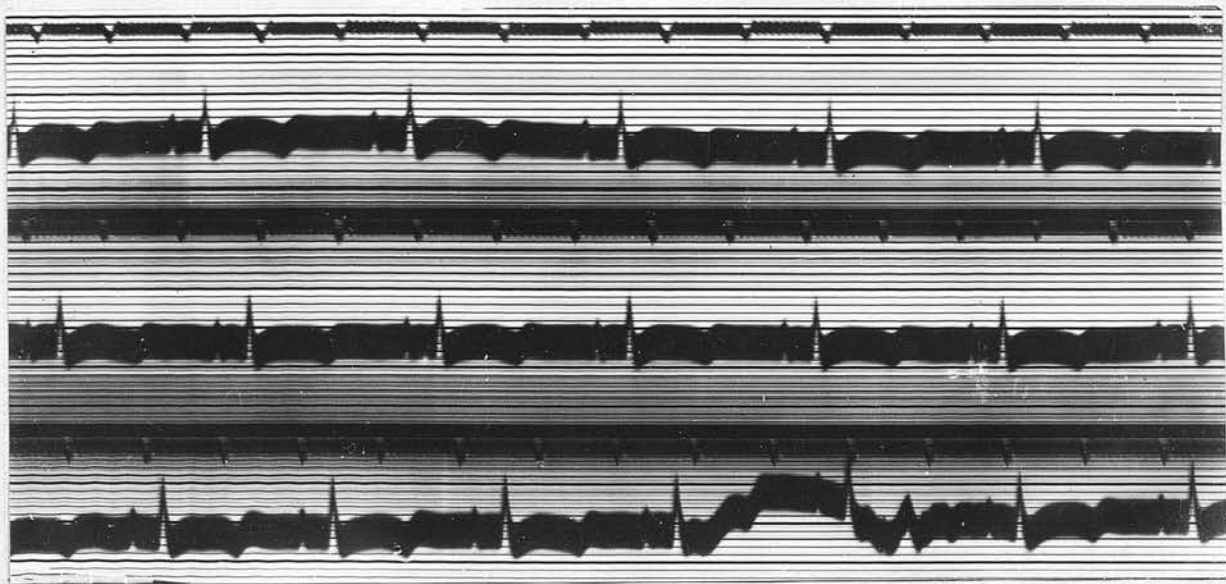


Fig. 32. 62 hour old embryo. The heart in this case was in contact with the electrodes. The heart rate in the above record is slower than normal because the egg was opened in the room. Read from left to right.

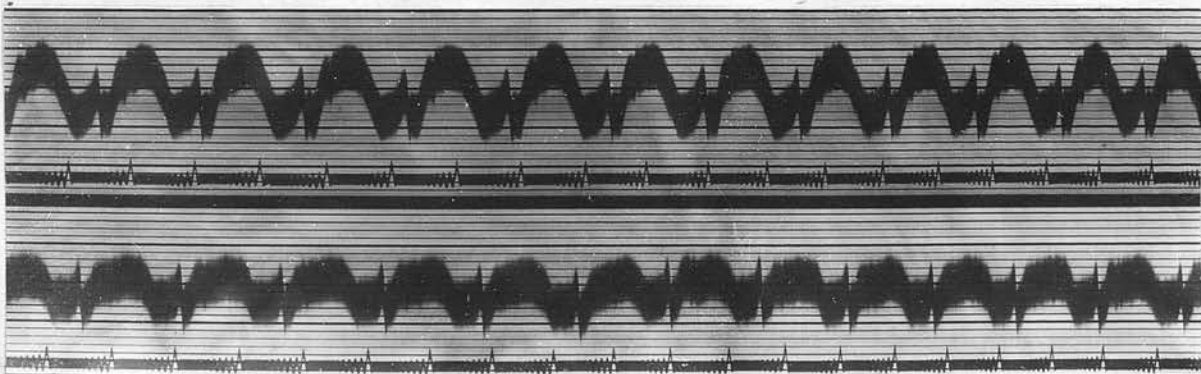


Fig. 33. 75 hour old embryo. Read right to left.

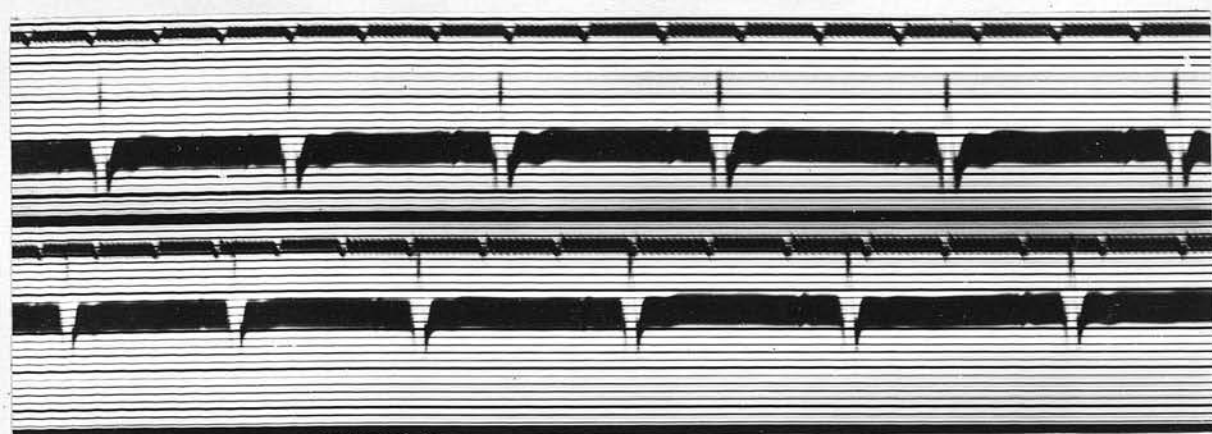


Fig. 34. 4th. day embryo. Egg opened, electrodes placed close to the heart. Resistance capacity coupling, amplifier after Adrian. Read from left to right.

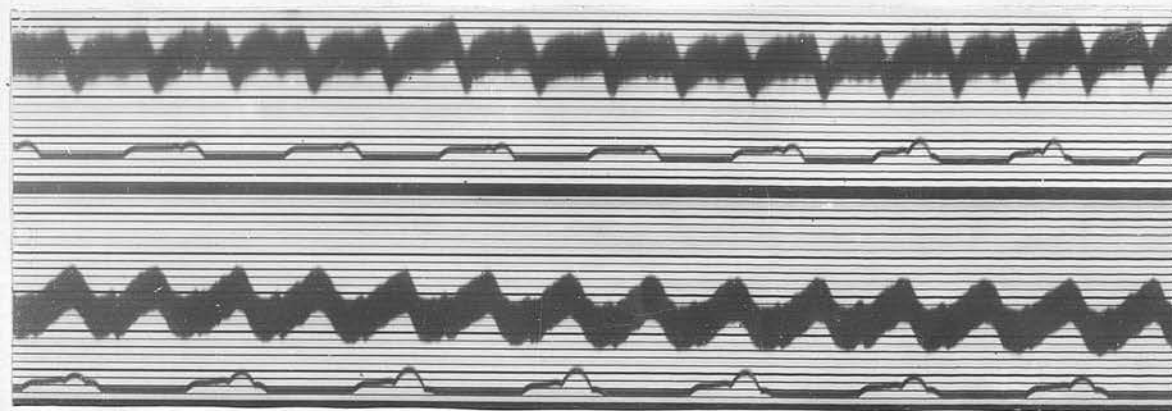


Fig. 35. 5th. day embryo.
Time in 0.50 seconds. Read right to left.

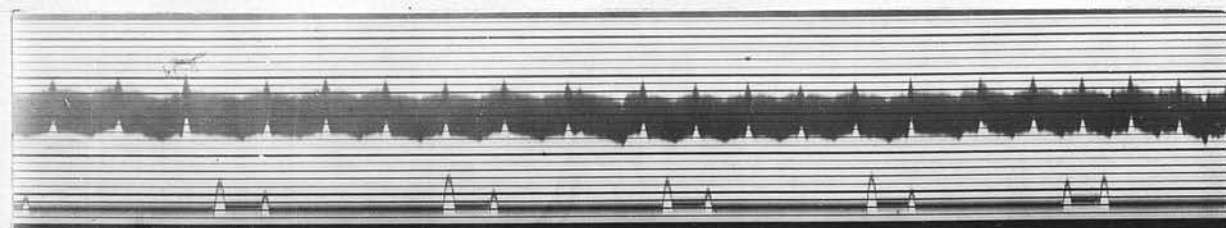


Fig. 36. 8th. day embryo.
Time in seconds. Read left to right.

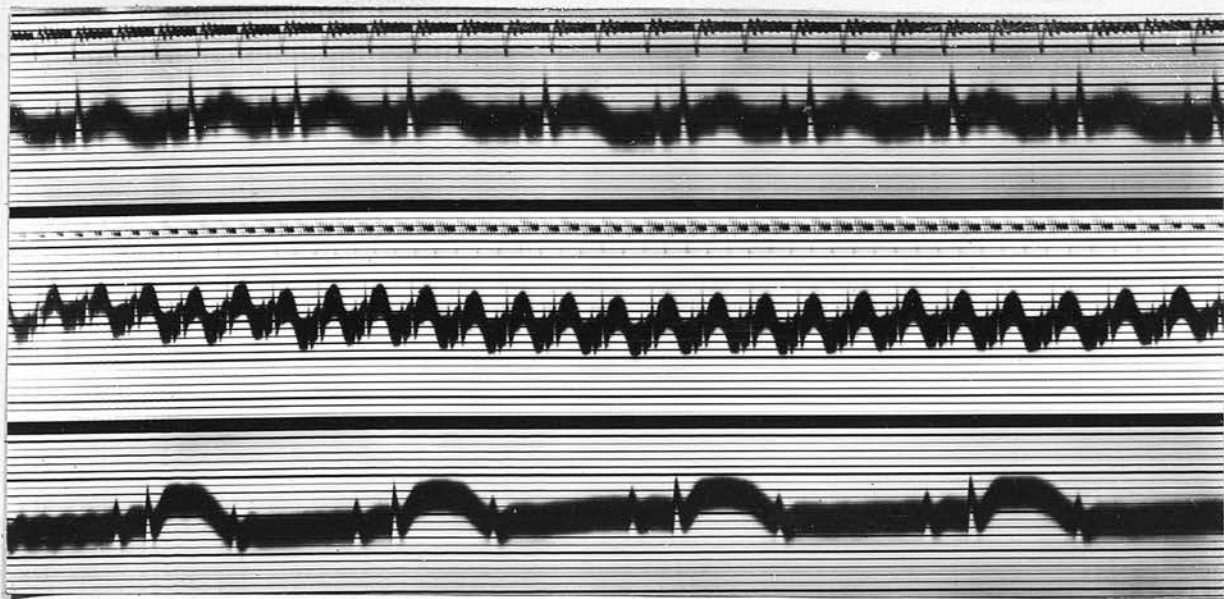


Fig. 37. 8th. day embryo, showing the development of a 2 : 1 heart block. One electrode was accidentally pushed into the body of the embryo, on dissection the auricle showed a small injury at the auricular ventricular groove. Read from left to right.

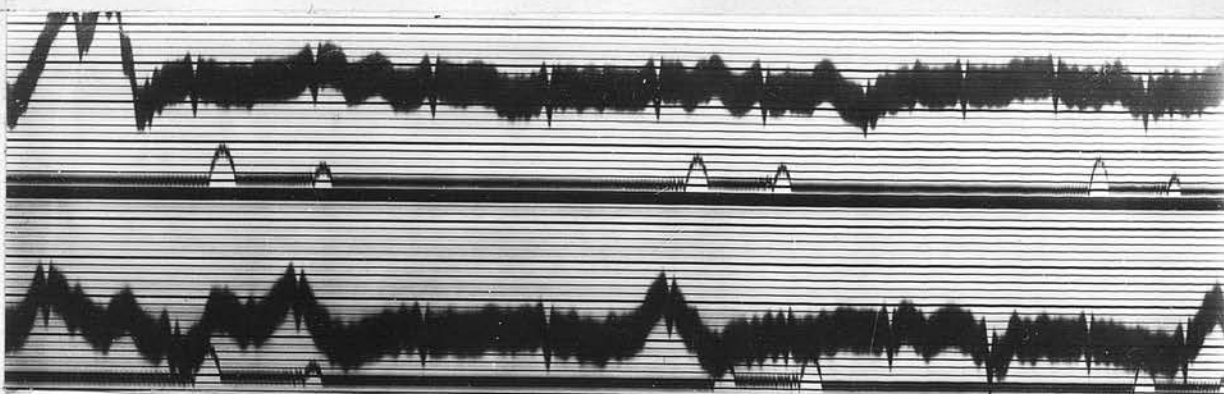


Fig. 38. 11th. day embryo. Unsteady base line due to movements of the embryo. Time in seconds. Read left to right.

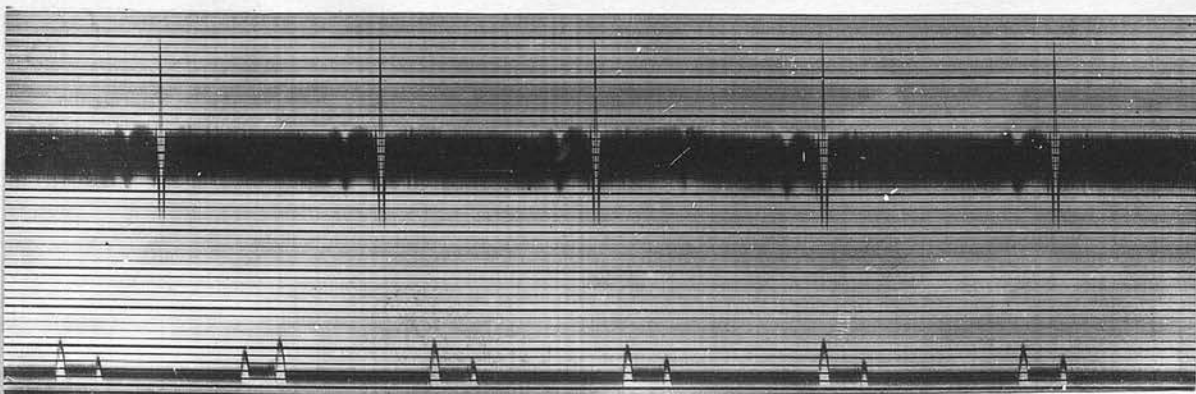


Fig. 39. Same embryo as in fig. 38., but after temperature had been allowed to drop to 17°C. Time in seconds. Read left to right.

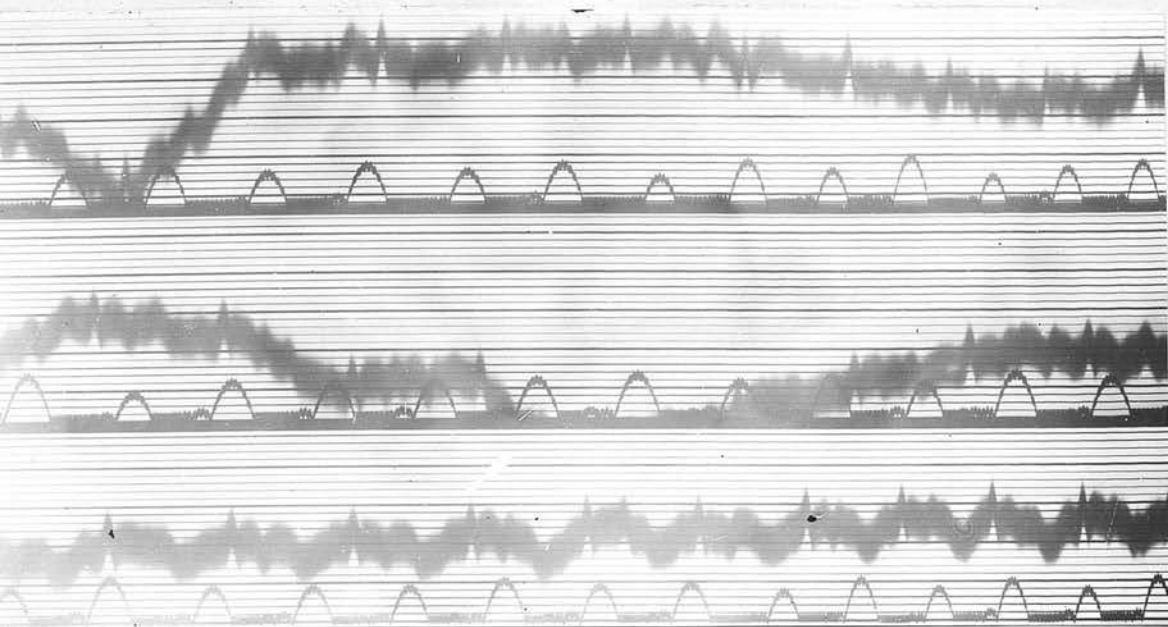


Fig. 40. 16th. day embryo. Unsteady base line due to movements of the embryo.

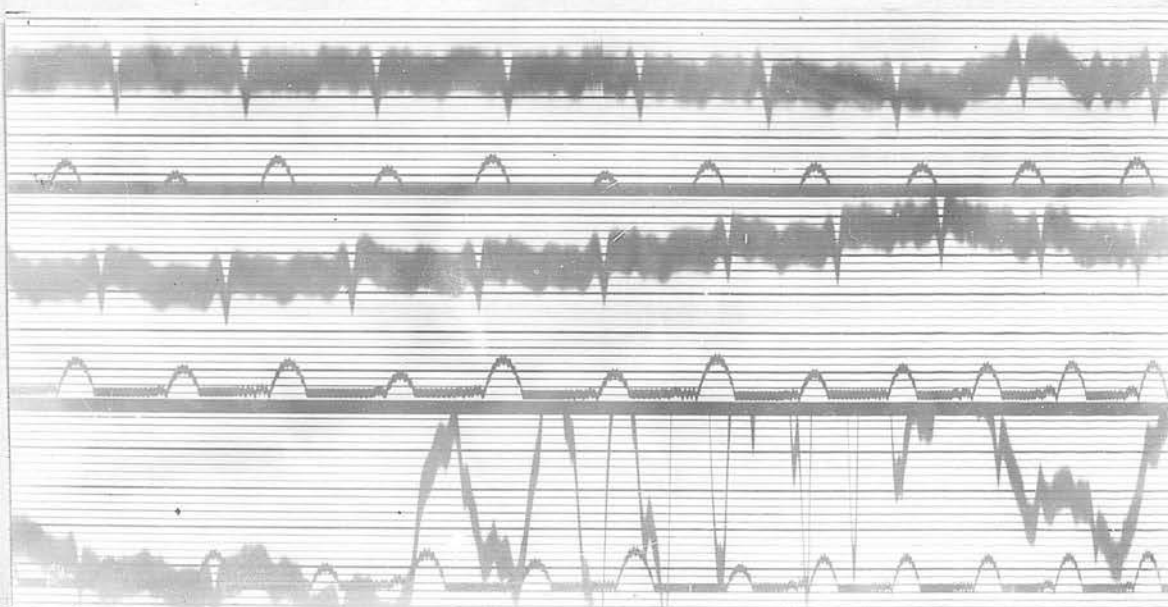


Fig. 41. 17th. day embryo. Note large excursions of string due to the kicking of the embryo.

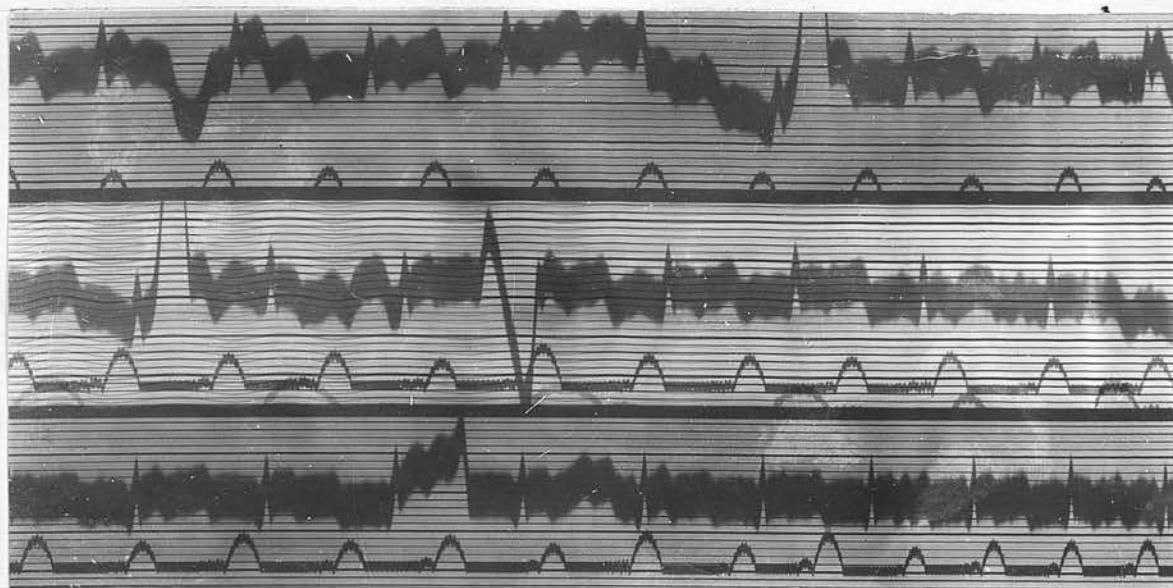


Fig. 42. 18th. day embryo.

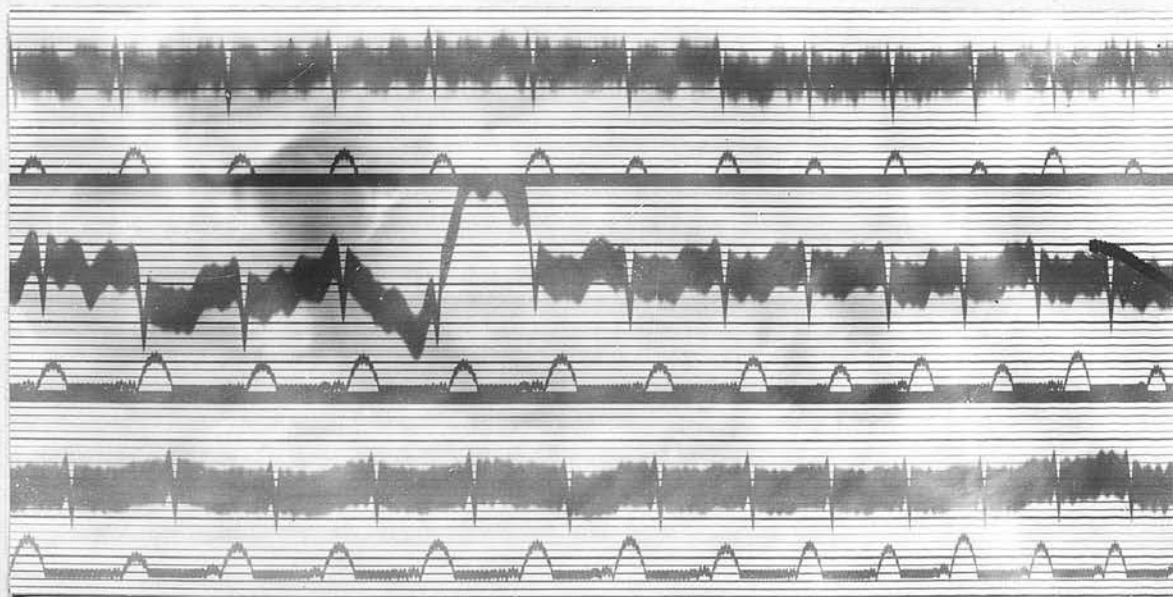


Fig. 43. 19th. day embryo.



Fig. 44.



Fig. 45.



Fig. 46.

Fig. 44. Microphotograph of a 2,3 day embryo showing size and position of the heart. Low power, x 36.

Fig. 45. Enlargement of the base of the heart shown in preceding photograph. High power, x 168.

Fig 46. Contact print taken from the slide of 4 serial sections of the embryo to show the actual size. There was a small amount of shrinkage due to mounting and fixing.

Table 1.

Age in days of incubation.	Beats per minute.	P-R interval in sec.	Duration of rise of R in sec.	Duration of R-T complex in sec.
2	120-140	-	0.009-0.012	0.28-0.32
3	180-210	0.07-0.09	0.08 - 0.01	0.20-0.21
4	195-210	0.10-0.11	0.01 - 0.016	0.12-0.13
5	200-240	0.09-0.10	0.01	0.21-0.24
6	205-240	0.10-0.13	0.01	0.25
7	200-235	0.08	0.01	0.22
8	205-240	0.05-0.10	0.01	0.17
9	205-240	0.094	0.01	0.18
10	210-240	0.12-0.13	0.01	0.11-0.28
11	240	0.11	0.01	0.11-0.28
12	210-240	0.089	0.01	0.18
13	210-240	0.10	0.01	0.18
14	210-240	0.11	0.01	0.14
15	240	0.053	0.01	0.15
16	240	0.068-0.083	0.01	0.14
17	240	0.059	0.009	0.14
18	240	0.056	0.013	0.14
19	215-264	0.050	0.009	0.11

Analysis of the electrical response of the developing chick embryo.

The above measurements were made with a Cambridge Instrument Company's comparator.

in all cases the records show a rapid and a slow deflection, and Figs. 24 to 28 suggest that both these two deflections are bi-phasic. Presumably the rapid deflection corresponds to the QRS waves and the slow deflection to the T wave of the adult electrocardiogram. The P wave was absent in all these records although in some cases there was a slight deflection, occurring from 0.15 to 0.175 seconds before the commencement of the rise of the R wave; this may or may not have been the P wave, since there was a certain amount of interference in these records due to external vibration, which could not be eliminated because at these ages the full amplification factor of the amplifier had to be utilised in order to obtain records without touching the heart. Figs. 29 to 43 show specimens of electrocardiographs obtained from embryos from the 2nd day of incubation until shortly before hatching.

As regards the general form of the electrocardiograph, the P wave did not appear until the 52nd hour and was always definite after the 60th hour of incubation. The electrocardiogram at the third day of incubation contains therefore the same components as does the adult electrocardiogram, as can be seen by comparing Figs. 32 and 52. Heart block could be produced after the 2nd day of incubation/

incubation either by rapid alterations in the temperature or by mechanical interference (cf. Fig. 37); this effect has been described by Johnstone (3) and Lewis (4).

The time relations of the electrocardiogram of the developing chick embryo at intervals of 24 hours are shown in Table I. The rate of rise of the R wave in the early embryo is the same as that of the adult, namely, 0.0009-0.01 seconds. The duration of the RT complex of the embryo during the second day of incubation, as measured from the beginning of the rise of the R wave to the end of the fall of the T wave, was a little longer than that of the adult (see Table 2), namely, 0.28-0.32 seconds. The heart rate during the second day of incubation varied between 120 and 140 per minute. This rate rose rapidly up to the 5th day when a maximum rate of 240 per minute was reached. There was very little variation in this rate up to the 19th day. On the 19th day the rate lay between 215 and 264 per minute. This slight fall was often noticed, but on the other hand, any slight alterations in the surroundings could cause an increase in the rate. Irritation of the embryo by/

by placing the electrode too far into the shell would often cause an increase in the rate. Very slight variations in temperature caused marked changes in the rate during the whole period of incubation.

My figures (e.g. Figs. 32 and 62) show a considerable resemblance between the electric responses of the heart of a chick at 60 hours incubation and that of an adult animal. In the former case, however, the heart is a point which is less than a millimeter in diameter. Its microscopical appearance is shown in Figs. 44, 45 and 46. These figures show that the heart wall is only about 20μ in thickness. It is interesting that the electric response of such a minute piece of tissue should resemble so closely the electric response of the adult heart.

The original purpose of these investigations was to determine whether there was any difference between the frequency of male and female embryos. For this purpose chicks with sex-linked plumage were used; this enabled the sex of the chick to be determined as soon as plumage appeared. The results were entirely negative, that is to say, no difference was observed between the average heart frequencies of the two sexes during incubation, and the same was found to be true in the case of chicks after hatching (cf. Section IV).

IV. THE ELECTROCARDIOGRAM OF THE CHICK.

Procedure.

The right wing and the left leg of the chick were cleaned with alcohol and ether, and a small strip of cotton wool soaked in warm saline was wound round the leg and base of the wing; only a very small pad was used so as to ensure the minimum of inconvenience to the chick. The wool was kept in position by means of fine silver wire coated with silver chloride. The chick was then placed in the oven at the same temperature as the brooder from which it had been removed and was allowed to rest for an hour on a pad of cotton wool, the oven being kept dark and well ventilated. If the cotton wool pads had dried during the resting period, they were moistened again and the chick allowed to rest for another quarter of an hour. Any interference such as light and noise tends to upset the chick and so make an appreciable difference in the rate of the heart beat. It was also/

also found that the chicks kept much quieter when several were placed in the oven at the same time with small cardboard partitions separating them.

Records were obtained in this manner from the moment of hatching to three weeks after. Three records were taken on the same plate at five minute intervals to ensure that the average rate thus obtained was as near normal as possible under these conditions.

The Heart Rate of the Chick.

In spite of the above precautions, the heart rate showed a considerable amount of variation, the lowest rate recorded being 157. This was due to the temperature of the oven being incorrect. The highest rate was 560, due to excitement when a 24 hour old chick was accidentally dropped. After 10 minutes the rate dropped to 450, 25 minutes after the fall the rate had dropped to 340-350 per minute (Fig. 48). There is no difference in the basal pulse rate of the male and female chick, but the male birds are somewhat more excitable and hence the pulse rate tends to show an increase. The average rate taken from over 200 records was 295 beats per minute for both the male and female. The rate varied under normal conditions between/

between 275 and 325 beats per minute. Any rate above or below these figures could be attributed to temperature variations, or to excitement of the chick.

Analysis of the electrocardiogram of the chick.

The electrocardiogram shows the usual complexes but with the P and T waves inverted. This is typical of the domestic fowl electrocardiogram. The following table shows the analysis of the various complexes of chick and adult as compared with those of a canary.

Table 2.

	P-R interval. sec.	Rate of Rise of R. sec.	Duration of Q.R.S. sec.	R.T. Interval sec.	Rate per minute.
Chick	0.05-0.06	0.004-0.006	0.018-0.20	0.08 - 0.13	295
Adult	0.06	0.009	0.02	0.13-0.16	290-300
Canary	0.04	0.0005	-	-	960
	0.05	0.0004 ⁽⁷⁾	0.015	0.0286	1,050

Figs. /

Figs. 47. to 51. show records obtained from the newly hatched chick to three weeks after. Temperature at which records were taken was 39°C . for chicks of 24 hours or less, and brooder temperature thereafter, which was $32-35^{\circ}\text{C}$. the first week, and $29-32^{\circ}\text{C}$. for the remainder of the time.

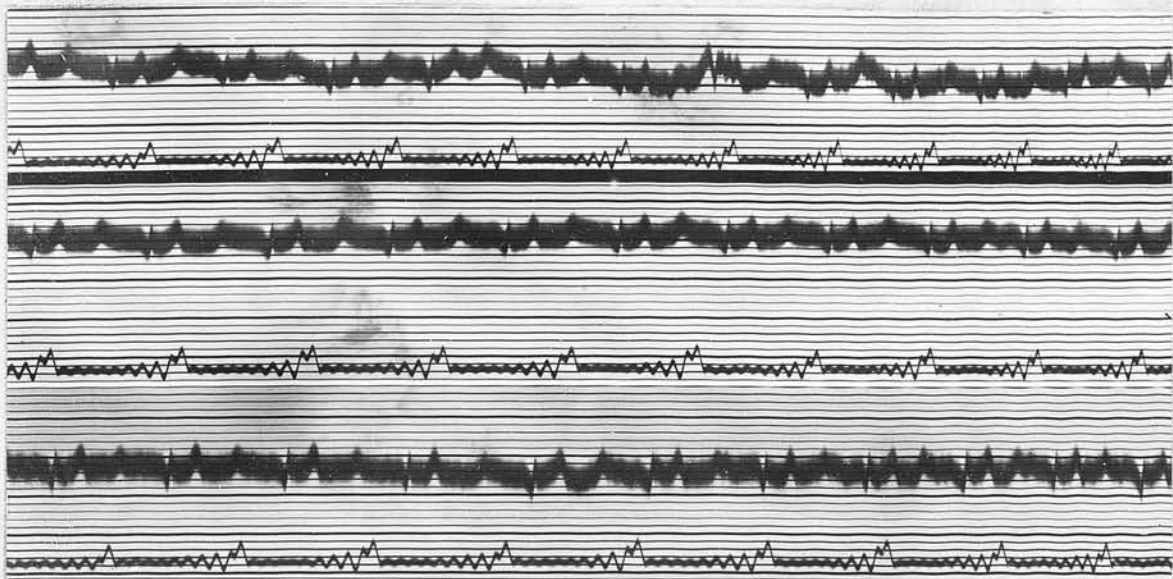


Fig. 47. Electrocardiogram of a chick 2 hours after hatching.
Read right to left.

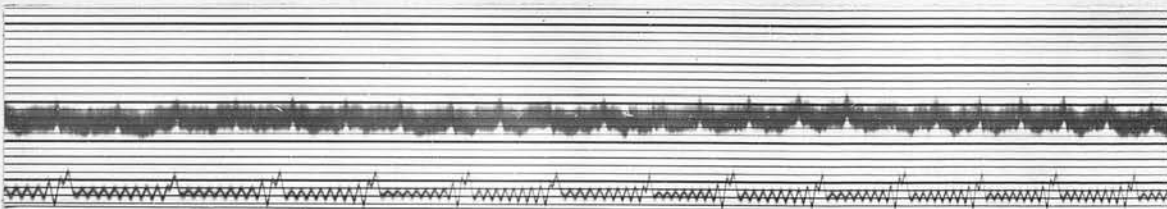


Fig. 48. 24 hour old chick accidentally dropped, heart rate increased to over 500 per minute. Read right to left.

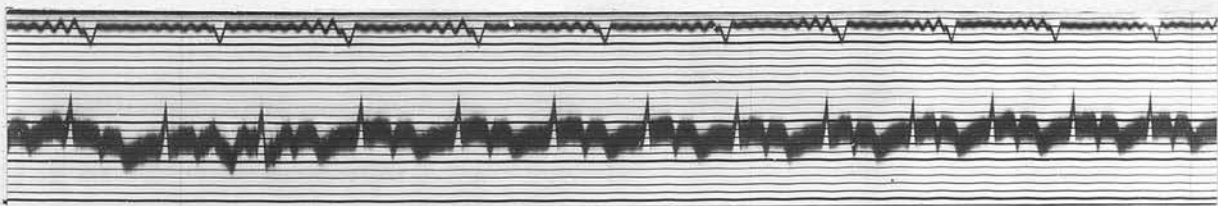


Fig. 49. Electrocardiogram of an 8 day old chick.
Read right to left.

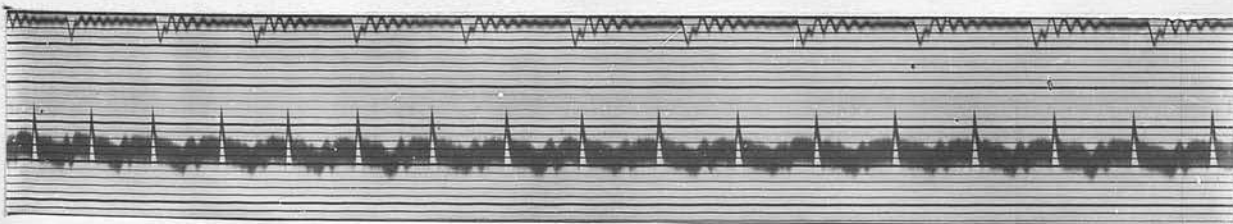


Fig. 50. Electrocardiogram of a 3 week old chick.
Read left to right.

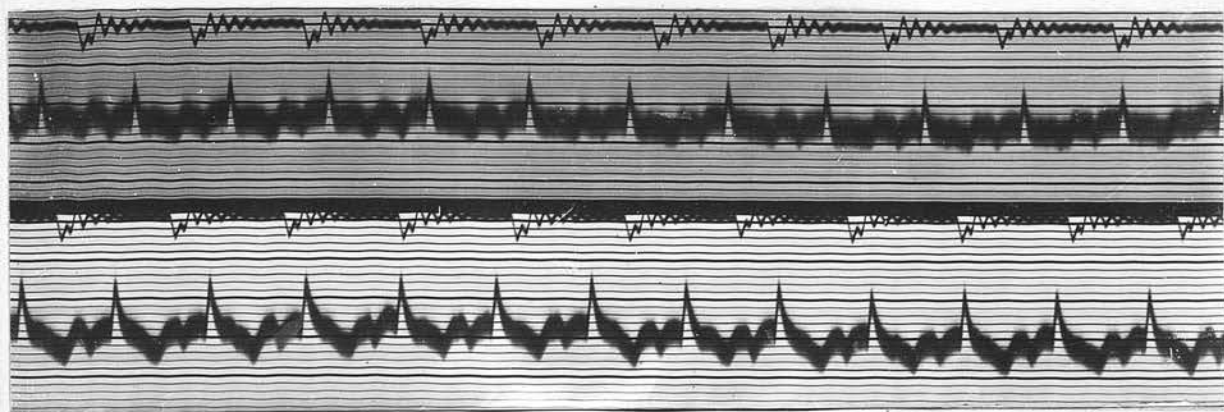


Fig. 51. Electrocardiogram of a 3 weeks old chick.
Read from left to right.

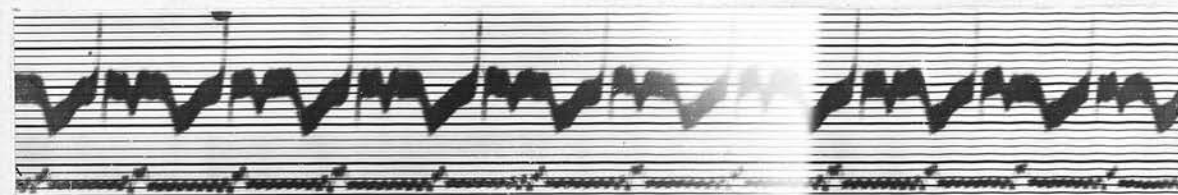


Fig. 52. Electrocardiogram of adult cock.
Read right to left.

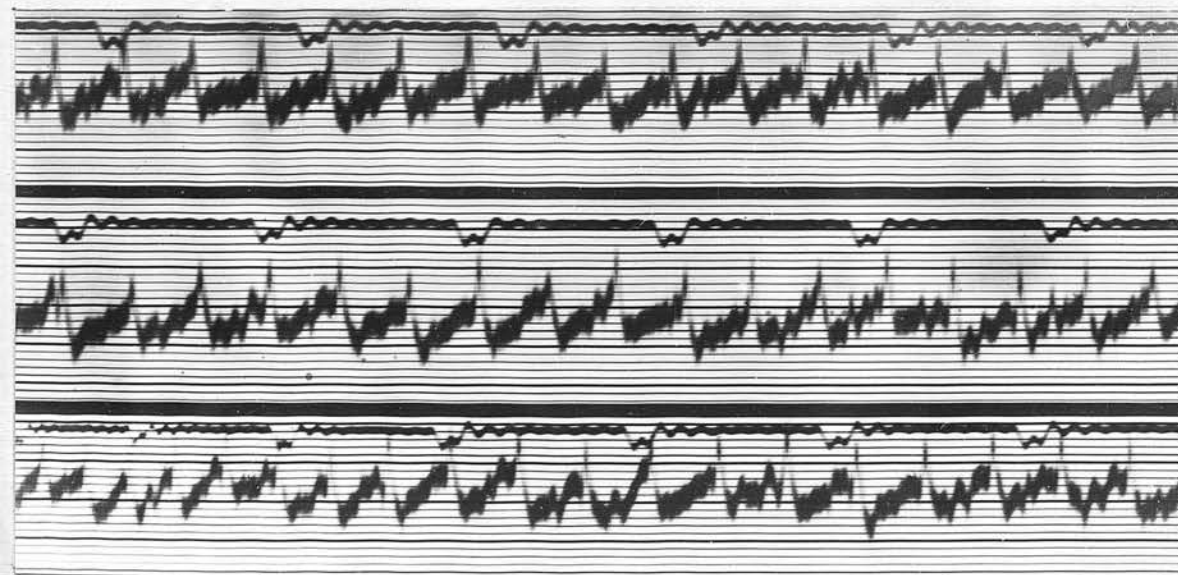


Fig. 53. Electrocardiogram of a canary.
Read from left to right.



V. THE ELECTRICAL RESPONSE OF SOME MARINE HEARTS.

Attempts were made to obtain records from the following species: (1) Mya, (2) Whelk, (3) Acanthia. Fresh specimens were obtained from the Marine Biological Station at Millport.

(1) Mya.

Procedure.

One half of the shell was removed with the minimum of interference; the umbo was then removed with a sharp pair of cutting pliers, and the pericardium was slit along its length thus exposing the heart, which was washed with sea water at frequent intervals. The electrodes were also kept moist with sea water.

Leads were taken from the base and apex of the heart, a very small piece of the wool of the electrodes being teased out and allowed to rest upon the heart. If this was not done there was distortion of the record owing to the movements of the heart under the electrodes. Silver wires coated/

coated with silver chloride were unsuitable since they produced distortion. Fig. 54. shows two records of the same heart: record (a.) was taken using silver wire electrodes and record (b) using wire covered with cotton wool.

Analysis of the Electrical Response.

No equivalent to the P wave was observed, but there was a definite R-T complex. The rate of rise of the R wave in relation to the whole complex is rapid, varying between 0.06 and 0.12 seconds. Fig. 56. however shows a longer rate of rise than was usual, namely, 0.48 seconds. The duration of the R-T complex varied between 2.0 and 2.5 seconds. The average rate of Mya hearts was found to be from 12-18 per minute. Since the heart is composed of plain muscle, this demonstrates that the rapid component of the complex is not an exclusive property of striped muscle, when the rate of rise of the R wave is taken relative to the duration of the R-T complex. Eiger (5) demonstrated a quick complex in the electrocardiogram of the oyster.

Fig. 55. shows the electrical and mechanical responses of the heart taken simultaneously. The latent/



Fig. 54. Electrocardiogram of the heart of Mya. Record (a) shows type of response obtained with silver wire electrodes resting on the heart, (b) the type of response when using wire covered with cotton wool. Time in seconds. Read from left to right.

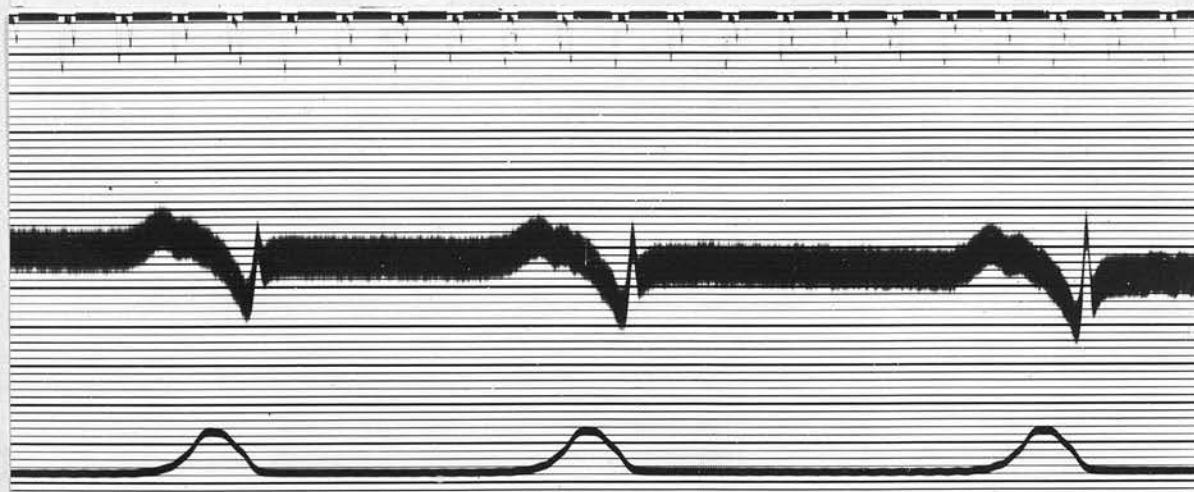


Fig. 55. Simultaneous mechanical and electrical responses of the heart of the Mya. Time in seconds. Read right to left.

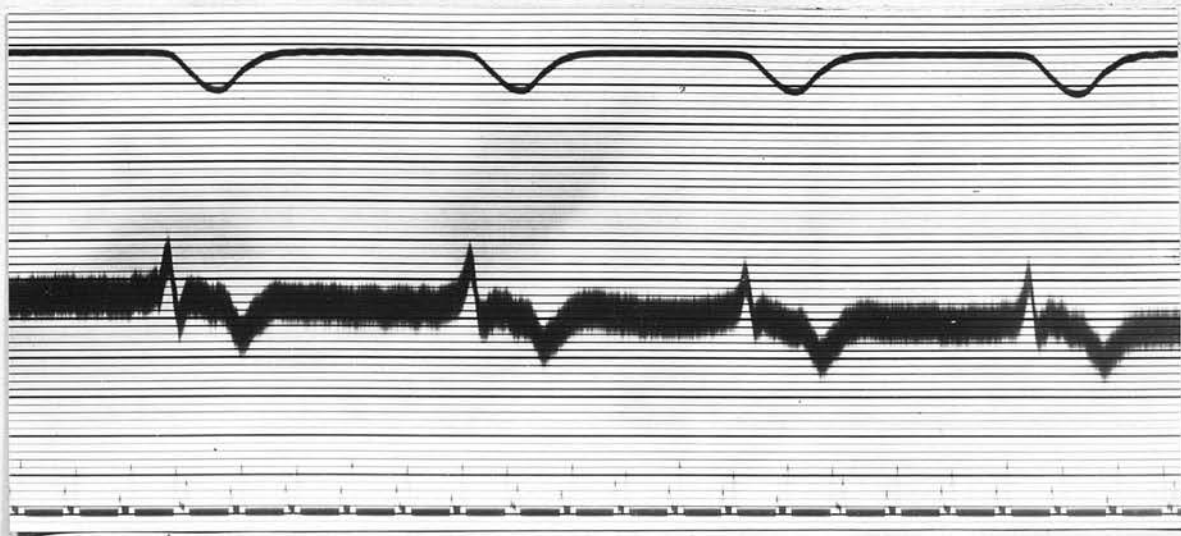


Fig. 56. Simultaneous mechanical and electrical responses of the heart the Mya. Time in seconds. Read left to right.

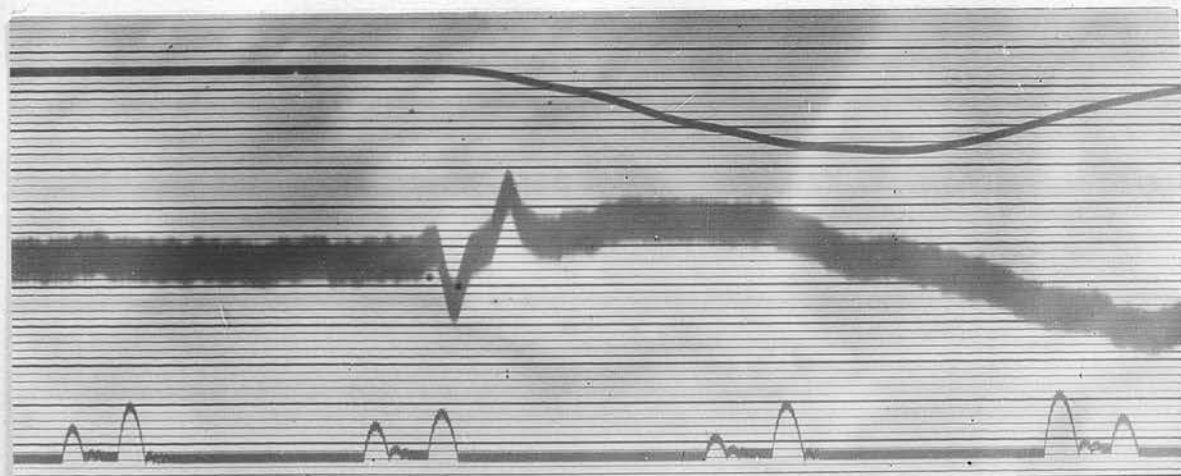


Fig. 57. Fast moving plate of the same heart as shown in fig. 56. Time in seconds. Read left to right.

latent period between the rise of the R wave and that of the mechanical response is 0.276 seconds, the rate of rise of the R wave is 0.117 seconds and the duration of the R-T complex 2.46 seconds. In order to record the mechanical response a very fine silver hook was inserted into the tip of the ventricle, and this was connected to the lever of a fine hair-spring myograph. The heart continued to beat for two days under these conditions as long as syphon was submerged in a beaker of sea water. Fig. 57. shows the form of the curve on a fast falling plate. The rate of rise of the R wave in this case was 0.055 seconds. A subsidiary phase was always noted in the T wave, which appeared to correspond to a secondary bulge in the ventricle occurring towards the end of the contraction phase. Attempts were made to take a simultaneous mechanogram and electrocardiogram to show the relationship between this phase and the electrical response (Figs. 55 and 56.) The insertion of the hook at the point where this phase normally occurred resulted in the obliteration of the phase or caused it to migrate to another portion of the ventricle. The average rate of the heart/

heart beat of the Mya was from 12-18 per minute.

(2) Whelk.

Some unsuccessful attempts were made to record the electrical response of the heart of the Whelk, but as soon as the heart was touched it went into a systolic spasm and no record could be obtained.

(3) Acanthia. - The Electrical Response of the Heart of young Acanthia.

The object of the experiment was to take the record of a heart whose action current was barely sufficient to produce a visible deflection of the string. Young Acanthia with their yolk sacs protruding from their body, probably about 15 to 18 months old, were found to suit the purpose.

Procedure.

The embryo was immersed in sea water containing 2.5 per cent. urethane, anaesthesia being complete in about 20 minutes. The lower jaw was then removed and the thoracic cavity opened to expose the heart. Silver electrodes coated with silver/

silver chloride and covered with cotton wool soaked in Ringer's fluid were placed lightly upon the base and apex of the heart. It was found that with a tension of 1 cm. to 0.2 of a millivolt there was only a very small deflection of the string (about a millimeter) using the galvanometer alone. With the AC/SG amplifier in circuit clear records were easily obtained with the string at full tension. Fig. 58 shows a record obtained with the string giving a deflection of 0.5 cm. with 1 millivolt. Analysis shows that there is a typical P.R.T. complex; the heart rate in this case was 32 per minute which is a little slower than the average under these conditions, namely, between 45 and 50 per minute. It was not possible to obtain a record from the unanaesthetised embryo owing to the difficulty in keeping it quiet and to the danger to the string since the mechanical movements were also amplified.

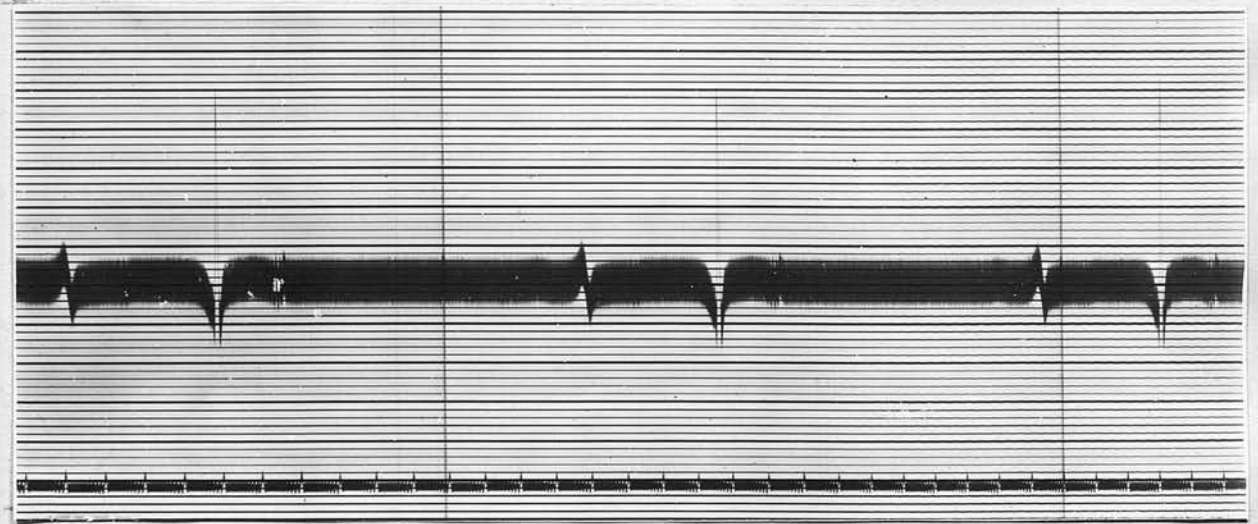


Fig. 58. Electrocardiogram of an *Acanthias* embryo.

VI. DISCUSSION.

(1) Pulse Rate of the Chick.

The contraction rate of the heart of the embryonic chick is very easily altered either by mechanical injury, or by exposure to cold. Most previous workers have failed to eliminate these errors and have given figures which are much too low.

Cohn (6) took adequate precautions and his figures for the embryonic heart rate agree closely with mine, as is shown in Fig. 58a, which gives the two sets of figures. The author's figures for each day show the maximum and minimum figures obtained from at least three separate measurements.

Fig. /

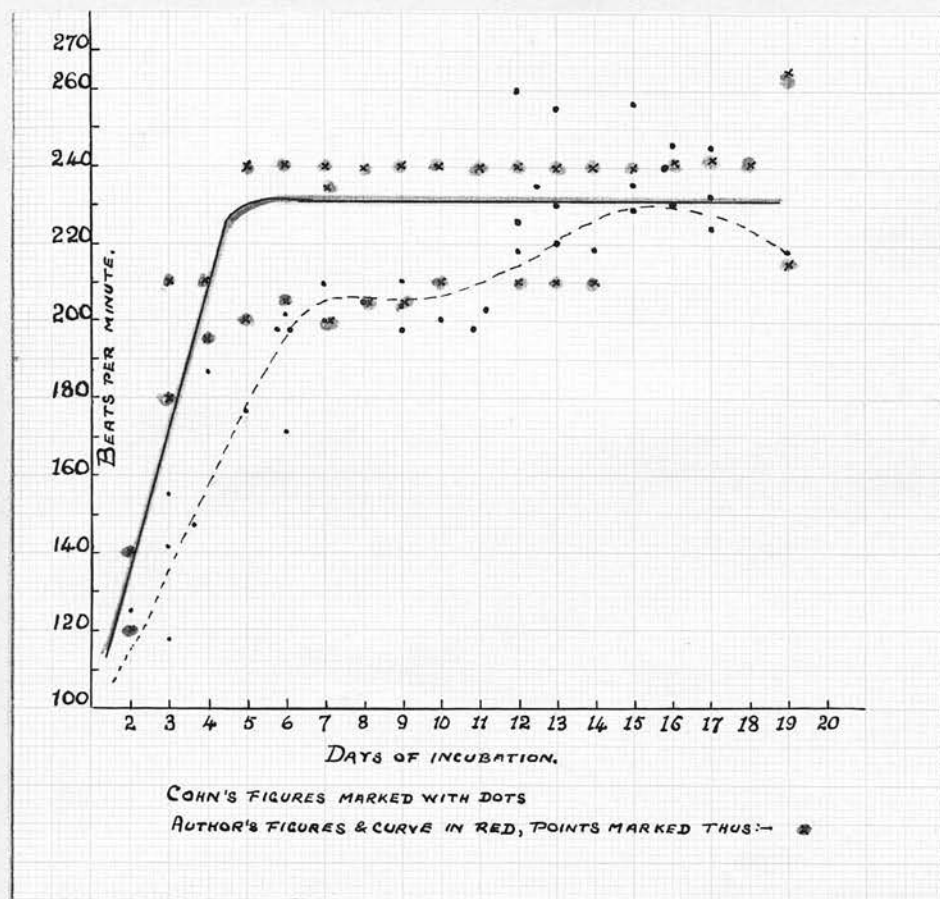


Fig. 58a.

The graph shows that the pulse rate rises rapidly during the first five days and then becomes remarkably uniform during the remainder of incubation. The only difference between the two sets of figures is that Cohn's figures show a somewhat slower rate of rise of pulse rate during the first week of incubation/

incubation. This difference is probably due to the fact that with the author's method it was not necessary to open the egg to obtain the record, and hence interference was reduced to a minimum. Cohn measured his rates by visual observation and this necessitated exposing the heart.

The following are a few figures of other workers:-

Age of egg in days.	Wernicke. 1876.(7)	Preyer. 1906. (8)	Pickering. 1893. (9).
2	90		85-138
3	142 (48-180)		92-140
4	150 (80-180)	123(101-139)	
6		128(86-150)	
8		148(139-154)	
11		167	

Lewis (4) noted that the heart rate after opening the shell varied between 120 and 214 on the second day and between 140 and 240 on the third day.

The pulse rate of the chick remains approximately constant between 200 and 240 per minute from the 5th day of incubation until hatching commences/

commences. The exertions during hatching cause an increase, and directly after hatching the average pulse rate rises to an average of 295 per minute and this rate is maintained practically constant throughout life, for the average pulse rate of an adult fowl at rest is only slightly more than 300 per minute.

It is of interest to note that in most other animals for which figures are available, there is a considerable difference between the rate in the adult and in the embryo. The heart rates of the embryos are higher than those of the adults, as is shown by the following figures quoted from Clark (10):

Table 3.

	<u>Foetus</u>	<u>New-born.</u>	<u>Adult</u>
Human	135 (5-9 month)	112	70
Oxen	161	141	50
Dog	120-170	160	100

Except in the case of the chick there are no figures for embryos at an early stage of development owing to the absence of any satisfactory method for obtaining such/

such measurements. A method, however, has been evolved by the author by which it is hoped to obtain reliable figures of foetal heart rates, when the technique is perfected. The method involves the use of a galvanometer which can be tuned into any desired frequency.

The curious constancy of the pulse rate of the hen during all stages of development may be due to the fact that the vagus exercises little tonic control over the pulse rate of the adult. Stübel (Clark, ref. 10, p.58) showed that in the adult hen vagal section only increased the pulse rate from 288 to 312. The writer found that injections of atropine into young chicks failed to show any rise of pulse rate that could be attributed to loss of vagal control. On the other hand, similar experiments on young ducklings showed a definite increase in frequency. Any excitement in the chick causes, however, a remarkable acceleration of the heart. Fig. 48 shows a case in which the pulse rate of a 24 hour old chick increased from a normal rate of about 300 up to 500 per minute.

The vagus therefore appears to exercise little tonic/

tonic control over the pulse rate of the chick or of the hen, whereas the sympathetic can produce a great acceleration of the heart. The pulse rate of the hen at all stages of development represents therefore the fundamental frequency of the pace-maker of the heart, which is but little affected by vagal control. In man on the other hand the low pulse rate (60-70) of the adult is due to vagal control and abolition of this control raises the pulse rate to a figure approaching that of the foetus.

In the duck the vagus exercises a powerful control, and from the analogy with man, the pulse rate of the duck embryo ought to be considerably greater than that of an adult duck. The author commenced experiments on duck embryos, but these were interrupted, and he hopes to complete them at a later date.

(2) Factors determining the Heart Frequency.

Clark (10) has shown that there is a close correlation in adult mammals and birds between the pulse frequency and metabolic rate. In such cases the blood pressure varies within a relatively narrow/

narrow range and the correlation can be explained by the following argument. The heart has to supply a certain quantity of blood per minute to the tissues in order to provide an adequate supply of oxygen. The minute volume equals the volume output per beat multiplied by rate per minute. As a first approximation the output per beat may be taken as varying as the size of the heart. In different animals the haemoglobin content of the blood does not vary extensively, nor does the amount of oxygen removed from a unit quantity of blood when the animal is at rest. Hence the minute volume may be taken as varying as the oxygen consumption.

Therefore Minute volume (\propto oxygen use) = Output per beat (\propto Heart weight) x frequency.

Therefore Frequency $\propto \frac{\text{oxygen use}}{\text{Heart weight}}$

Since metabolic rate = $\frac{\text{oxygen use}}{\text{body weight}}$ and heart

$$\text{ratio} = \frac{\text{Heart weight} \times 100}{\text{body weight}}$$

Therefore Frequency $\propto \frac{\text{Metabolic rate}}{\text{Heart ratio}}$

It is of interest to see whether this general rule applies when the embryo chick is compared to the adult hen. The author obtained the following figures/

figures for the heart ratios.

Age of embryo in days	Body weight in mgm.	Heart weight in mgm.	Heart ratio.
3	65	1.2	1.8
4	82	1.8	2.2
4	333	2.8	0.87
5	116	4.8	4.2
6	336	7.0	2.1

Unfortunately these figures are extremely variable as it is very difficult to isolate such minute pieces of tissue. They indicate, however, that the heart ratio of the embryo is about 2.0. The heart ratio of an adult hen is 0.68 (Clark, ref. 10, p. 78).

The following figures have been obtained for the metabolism of young chick embryos.

Figures/

Figures from Bohr & Hasselbach (11).

Age of embryo in days —	4	5	6	8
Weight of embryo in mgm. —	60	130	360	1000
CO ₂ production in c.c. per diem —	6.1	10.6	17.9	29.6
Calories per kilo per diem (1 litre CO ₂ = 6.7 calories) —	685	540	340	200

Figures from Murray (12 and 13).

Age of embryo in days —	5	6	7	8
Weight of embryo in mgm. —	221	423	735	1189
C.c. CO ₂ per gm. per diem (12) —	29.2	30.1	28.5	29.8
Cc.O ₂ do. (13) —	-	50	43	39
Calories per kilo per diem. RQ = 0.7, hence 1 litre CO ₂ = 6.7 cals. —	194	202	190	200
1 litre O ₂ = 4.7 " —	-	235	202	183

These figures do not show a good agreement, but the approximate metabolic rate for a chick embryo of 5 to 6 days with a weight of 300 mgm. appears to be between 250 and 350 calories per kilo per diem, and 300 calories may be taken as an average figure.

The/

The metabolic rate of an adult hen which weighs 2000 gm. is about 65 calories (Clark, ref. 10, appendix 1). These figures enable us to apply the formula:

$$\text{Frequency} \propto \frac{\text{Metabolic Rate}}{\text{Heart ratio}}$$

In the case of the embryo chick of 5-6 days,

$$\frac{\text{metabolic rate}}{\text{heart ratio}} = \frac{300}{2} = 150, \text{ whereas in the case of}$$

$$\text{the adult fowl the figure is } \frac{65}{0.68} = 94.$$

According to this formula the frequency of the chick embryo heart should be one and a half times that of the adult heart, whereas it is approximately the same. This calculation shows therefore that weight for weight the embryonic heart is more efficient than is the adult heart in providing oxygen to the tissues. The explanation for this is probably that the pressure against which the hearts work is very different in the two cases.

The blood pressure in the adult hen is about 150 mm. mercury, whereas the blood pressure in the chick 2-3 days old is only 1.5 to 2.0 cm. of water. (Hill and Azuma, 14). Since the work of the heart varies as volume output multiplied by resistance, hence the heart of the embryo, which works against an/

an almost negligible pressure, can maintain a far greater output per unit of weight than can the adult heart. The similarity between the pulse rates in the embryo chick and in the adult hen appears therefore to depend on the fact that in the chick the volume output per beat per unit of heart weight is considerably greater than is that of the adult hen. This conclusion is supported by Figs. 45 and 46, which show that the walls of the heart of the chick embryo are very thin.

(3) The P-R Interval.

The P-R interval measures the time between the moment the wave of excitation invades the auricle and the moment it invades the ventricle. The interval must measure the sum of three components, namely, the times taken for the wave to pass firstly from the auricle to the A-V node, secondly through the A-V node and thirdly from the A-V node to the ventricle. The relative duration of these components is a matter of dispute. The length of the P-R interval is influenced by the frequency, for increase of frequency increases its duration.

Clark/

Clark (ref. 10, p.50-51) noted that there was remarkably little difference in the P-R interval of mammals of varying size, for instance the P-R intervals of the elephant (2,000,000 grams) and of a small bat (5 grams) were respectively 0.3 and 0.03 seconds. In this case, however, there were two variables, namely, pulse rate and size, for the elephant was 400,000 times the weight of the bat, but the heart rates were 40 and 660 per minute respectively.

The adult hen and the chick embryo provide an example of two hearts which differ greatly in size but which contract at a similar rate. The following figures show that the P-R interval is almost constant throughout the development of the hen.

Age	Heart Rate	P-R Interval	Length of Heart.
5th day embryo	230	0.09 sec.	2.0 mm.
5th day chick	295	0.06 sec.	14.0 mm.
Adult cock	320	0.06 sec.	40.0 mm.

The distance from the pace-maker to the ventricle must vary as the length of the heart, and consequently the wave of excitation must travel about thirty/

thirty times as fast in the adult animal as in the young embryo. My figures for the P-R interval would give a rate of about 18 mm. per second for the passage of the wave of contraction from one end of the heart to the other. This agrees fairly well with the results of Fano and Badano (15) who measured the mechanical response of the heart of the chick embryo and found rates of conduction of from 3.6 to 11.5 mm. per second. The difference between the figures may well be accounted for by the mechanical interference necessitated by Fano and Badano's method, which probably reduced the rate of conduction in their experiments. My earliest records of the P-R interval were obtained during the 2nd day. Figures 44, 45 and 46 show a section of a heart in which a P-R interval of 0.08 sec. was recorded just before the embryo was killed, although no P wave could be detected six hours previously. The section shows that the heart is in the form of a convoluted tube. The actual measurements of the heart were as follows: length 0.61 mm. and breadth 0.16 mm.

Kulbs (16) noted that fatigue changed the polarity of the P wave. I was able to confirm this, but the polarity was not always the same in/

in different hearts, in different stages of development. Later however the wave was usually inverted as in the chick and adult. Spadolini and Georgio (17) noted a P wave at the 50th hour, the P-R interval being 0.1-0.15 seconds with a heart rate of 70 per minute, which is very much lower than normal. Wertheim Salomonson noted a P-R interval of 0.1 seconds on the 8th day of incubation.

(4) The Form of the Ventricular Complex.

Wertheim Salomonson (18) and Cluzet and Sarvonat (19) suggested that the form of the electrocardiogram commenced as a simple wave which became more complex as development progressed. Using the same tension for the string as Salomonson, namely 25 cm. for 1 millivolt, it was possible to obtain similar curves to those shown in Fig. 59, see Fig. 60. This suggests that the form of curve obtained by these workers was due to the employment of a very loose string; they were compelled to do this as they did not have the thermionic valve at their disposal. My own work shows that the earliest record is made up of a fast and a slow component, i.e. an R-T complex. The rate/

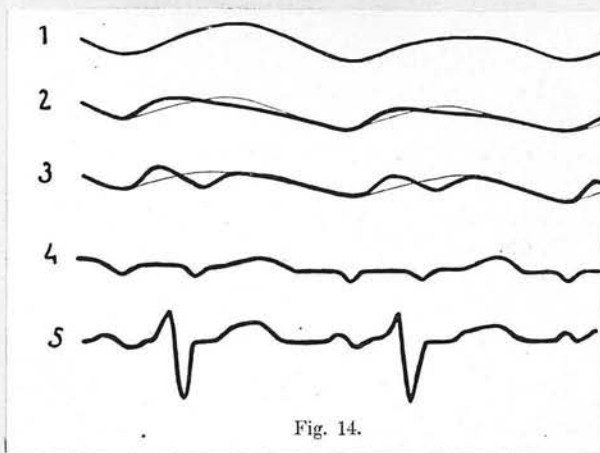


Fig. 59. Diagrammatic drawings showing the development of the electrical complexes of the developing chick embryo heart. (From Wertheim Salmonson.)

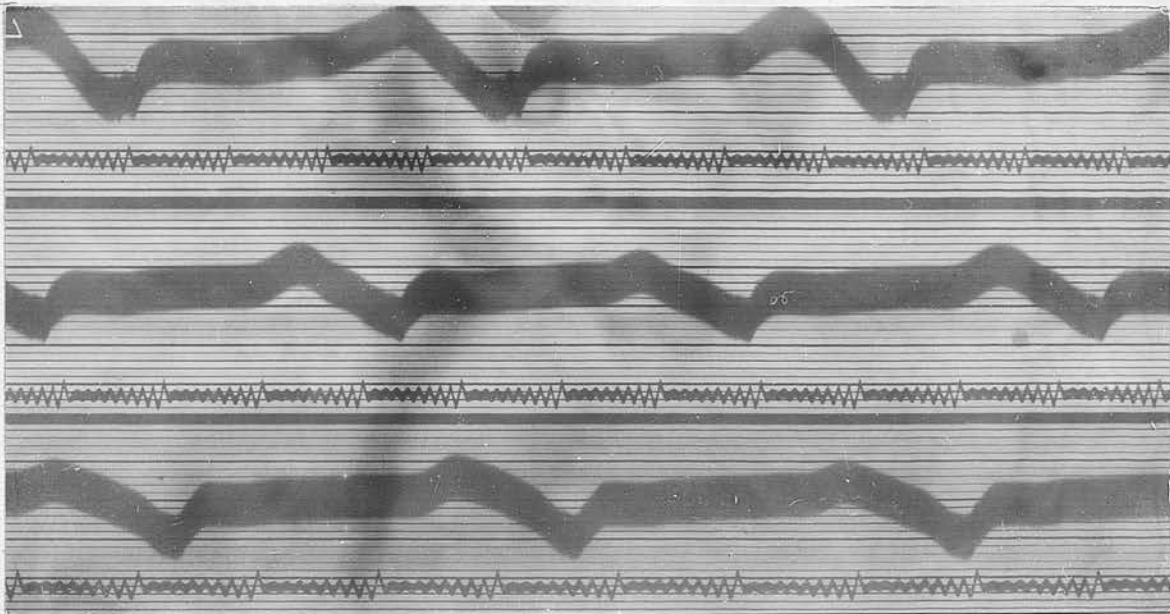


Fig. 60. Type of curve obtained when a very slack string and no amplifier was used, the tension was 25 cms. for 1 millivolt. Note the similarity of these curves to those in fig. 59. Embryo of 3.5 days.

rate of rise of the R wave is somewhat longer in the earliest record than in the adult (cf. Table 2).

There is remarkable similarity in the time relations between the early embryo and the adult; in fact the electrocardiogram of the 3rd day embryo is practically identical with that of the adult. This similarity is very remarkable in view of the fact that the heart of the adult hen is several hundred times as large as that of the young embryo, and is at least twenty times as long. Apparently all conduction processes (as indicated by the P-R interval, and by the duration of rise of P and of R waves) when calculated in distance per second are about twenty times slower in the young embryo than in the adult hen, and hence the difference in size is compensated for by the difference in conduction rate and the curious similarity in electrical response results. This difference in the rate of conduction is all the more remarkable because the frequency of the pace-maker is the same in the two cases.

VII. SUMMARY.

(1). A method is described by which it is possible to study feeble action currents.

(2). Various forms of distortion and interference are discussed.

(3). A complete analysis of the electrical response of the hen embryo is demonstrated.

(4). The heart rate throughout the period of incubation has been established under as nearly ideal conditions as possible.

(5). The earliest record of electrical response of the heart of the embryo showed a fast and a slow component making up a QRST complex.

(6). The electrocardiogram of a 3rd day embryo was practically identical with that of the adult.

(7). The rate of conduction in the embryonic heart was very slow.

(8) /

(8). Figures are given for the heart rate of the newly hatched chick during the first three weeks; these figures are the same as those of the adult.

(9). The newly hatched chick showed remarkable accelerator action of the sympathetic.

(10). There was little evidence of vagal control in the chick, but very definite control in the duckling.

(11). Very slight variations in temperature had a marked effect on the heart rate of the embryo and of the newly hatched chick.

(12). Electrocardiograms and mechanograms were taken of the *Mya* heart; a fast and a slow component was demonstrated.

(13). An unsuccessful attempt was made to record the electrical response of the *Whelk*, but the heart went into systole as soon as it was touched.

(14). The electrical response of the *Acanthia* embryo was also recorded.

(15) /

(15). The theory that early embryo hearts gave an electrical response consisting of a simple wave which became complex as development progressed, is discussed, and the curves imitated.

I wish to take this opportunity of thanking Professor Clark for his invaluable help and criticism. It was also due to him that I was able to procure the necessary equipment and accommodation.

Part of the expenses were also defrayed by grants from the Moray Fund.

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